The triple neurokinin-receptor antagonist CS-003 inhibits neurokinin A-induced bronchoconstriction in patients with asthma

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Abstract:

Neurokinin A (NKA) causes bronchoconstriction in asthmatic patients. In vitro both NK_1 and NK_2 receptors can mediate airway contraction. Moreover in guinea pigs, NK_3 receptors facilitate cholinergic neurotransmission. Dual tachykinin NK_1/NK_2 receptor antagonism results in prevention of NKA-induced bronchoconstriction. We have now examined the effect of a single dose of the triple tachykinin receptor antagonist CS-003 on NKA-induced bronchoconstriction in asthmatics.

A double blind, crossover, placebo-controlled trial in 16 mild asthmatics was performed. One single dose of CS-003 (200 mg, solution in distilled water) or matched placebo was given orally on the assessment days. NKA-provocation tests were performed pre-dose and 1,8 and 24 h after dosing.

There was a significant shift to the right of the dose-response curve at 1 and 8 h after intake of CS-003. PC_{20} was not reached in 12/16 patients at 1 h post-dose and in 5/16 patients at 8h post-dose. This did not occur under placebo treatment.

A single dose of 200 mg CS-003 protected significantly against NKA-induced bronchoconstriction at 1 and 8 h post-dose in mild asthmatics.

Keywords: Asthma; Neuropeptides; Neurokinin A; Bronchoconstriction; Tachykinin receptor antagonist

1. Introduction

Neurokinin A (NKA), Neurokinin B (NKB) and substance P are members of the tachykinin peptide family. They are present in sensory nerves and in immune cells [1]. SP binds preferentially on the tachykinin NK_1 receptor, whereas NKA binds on the NK_2 and NKB on the NK_3 receptor. But they can interact with all three receptors. In the human lungs, tachykinins increase mucus secretion from the glands, increase permeability of the blood vessels resulting in plasma leakage and vasodilatation. They attract and activate inflammatory cells [2-4]. Binding of the receptors in the smooth muscles causes bronchoconstriction. Finally they facilitate cholinergic neurotransmission. Of all these effects, bronchoconstriction is the most studied effect [5]. Asthmatic [6] patients are more sensitive to the bronchoconstrictor effect of substance P and NKA [7-9]. Studies on isolated human airways have shown that tachykinin-induced bronchoconstriction is mainly caused by activation of the tachykinin NK_2 receptor [7]. However, contraction of the small- and medium-sized human bronchi can also be mediated by tachykinin NK_1 receptors [10,11]. Studies on guinea pig and human airways show that NK_3 receptors facilitate cholinergic neurotransmission [12-15]. Therefore, agents blocking all three receptors may be useful in the treatment of asthma.

There are limited data available from clinical trials with tachykinin antagonists in asthma. FK 224, a dual tachykinin NK_1/NK_2 antagonist caused a poor protection against NKA-induced bronchoconstriction. Although active in guinea pigs, it did not protect against NKA-induced bronchoconstriction in patients with asthma [16]. SR 48968 (saredutant) and MEN 11420 (nepadutant), both tachykinin NK_2 receptor antagonists, caused a small, but significant inhibition of the NKA-induced bronchoconstriction in asthmatics [17,18]. DNK 333, a dual NK_1/NK_2 receptor antagonist protected clearly against the NKA-induced bronchoconstriction in mild-to-moderate asthmatics. Although DNK 333 had a good activity in the NKA-provocation model, the duration of action of this compound was rather short [19]. Moreover, the tachykinin NK_3 receptor plays an important role in the pathogenesis of asthma, especially for cough and hyperresponsiveness [12]. Therefore, a triple NK-receptor

antagonist could be a more efficient approach in blocking the tachykinin receptors in the airways.

CS-003 is the first potent triple neurokinin receptor antagonist. In vitro as well as in vivo antagonistic activity against tachykinin NK_1, NK_2 and NK_3 receptors has been shown. In guinea pigs CS-003 inhibited substance P-induced tracheal vascular hyperpermeability, NKA-induced bronchoconstriction and NKB-induced bronchoconstriction [20].

The aim of the present trial was to examine the effect of CS-003 on the NKA-induced bronchoconstriction in mild-to-moderate asthmatics.

2. Materials and methods

2.1. Study design

This was a randomised, double blind, placebo-controlled, two-way crossover trial. The trial was performed in two different university hospital departments of respiratory diseases. The protocol was approved by the ethical committees at each of these centres.

2.2. Patients

Both female and male patients between 18 and 50 year old with stable mild-to-moderate asthma were eligible for the trial. All patients gave written informed consent before entering the trial. Patients were only allowed to use short-acting β-agonists as concomitant medication. At screening, their morning forced expiratory volume in 1 s (FEV_1) had to be equal or greater than 75% of their predicted value. All patients had a provocative concentration of methacho-line producing a 20% fall in FEV_1 (PC_{20}) ≤8mg/mL at screening or within 1 year of screening. At the first or second screening visit (depending on the necessity of the methacholine provocation), all patients underwent an NKA-provocation test, their PC_{20} had to be ≤$10^{-6.5}$mol/mL to be included. All patients were nonsmoking, normotensive and had a normal body mass index. They were excluded if they had a recent respiratory tract infection (within 4 weeks of screening) or an asthma exacerbation leading to hospitalisation within 6 months prior to the study. Other exclusion criteria were clinically significant laboratory abnormalities, other systemic diseases, history of alcohol or drug abuse.

2.3. Study protocol

The trial consisted of a screening visit, 2 one-day treatment periods (each over 24h) and a follow-up visit. There was a washout of 3-14 days between each treatment period. Patients were requested to withhold their short-acting β_2-agonists for at least 6h before each visit to the site.

For each trial period, each patient was admitted to the hospital from the evening before drug dosing until 24h after drug dosing. During treatment period 1, the patients were randomised to receive a single dose of CS-003 (200 mg, solution in distilled water, orally) or placebo. The patients received the alternative treatment in the second period. All evaluations performed were equal for both periods. Administration of the drug was in the morning after an overnight fast. Before administration, an ECG was performed as well as a physical examination (with vital signs) and a blood sample was taken for routine laboratory tests and pharmacokinetic analyses pre-dose. FEV_1 was measured together with an NKA-provocation test at pre-dose, 1, 8 and 24 h after administration. On day 2 of the same treatment period, we checked the patient for adverse events and/or use of concomitant medication. Again an ECG was performed and vital signs were measured. A blood sample was taken for routine laboratory tests. Pharmacokinetic samples were planned immediately after the NKA challenge at 1, 8 and 24 h post-dose.

2.4. Pulmonary function testing

The FEV_1 was obtained using flow-volume loops with a pneumotachograph (Vmax 20C, SensorMedics, Yorba Linda, California, USA). The highest value of three consecutive manoeuvres was accepted for evaluation at each performance.

2.5. Bronchial challenge tests

At screening, a methacholine challenge was performed for patients for whom a PC_{20} FEV_1 for methacholine ≤8mg/mL was not documented within 1 year before screening. The PC_{20} for methacholine was determined by

measuring the decrease in FEV_1 after inhalation of doubling doses of methacholine.

Before starting the NKA provocation, we measured a pre-challenge FEV_1 (= baseline). The challenge was started after inhaling the diluent solution. When the post-diluent FEV_1 did fall less than 10% compared to the baseline FEV_1 the challenge could be continued with increasing doses of NKA ($10^{-8.5}$, 10^{-8}, $10^{-7.5}$, 10^{-7}, $10^{-6.5}$ and 10^{-6} mol/mL) until FEV_1 dropped 20% compared to the post-diluent value. The measurements of FEV_1 were performed 3 and 7 min after the start of each inhalation, with the lowest value of each being considered as the value.

NKA (Bachem, Bubendorf, Switzerland) was diluted in saline containing 1% human serum albumin (Behringwerke, Marburg, Germany). The dilutions were made on the morning of the visit and were kept on ice until the provocation started. The aerosols were produced using a Mallinckrodt jet nebuliser (Mallinckrodt Diagnostica, Petten, The Netherlands). A collapsible bag of 30 L was filled with nitrogen, then 0.5 mL of diluent or each subsequent NKA concentration was sprayed by compressed N_2 in 60 ± 10 s in this bag where the droplets were evaporated to dry particles. Patients inhaled the aerosol from the bag in 2 min by quiet tidal breathing through a 3-way valve and mouthpiece, until the bag collapsed. They were in sitting position with their nose closed by a clip. Supplementary oxygen was supplied in the mouthpiece (at a flow rate of 4L/min, inspiratory oxygen fraction [FIO_2] = 0.995). The provocation continued until there was a drop of 20% or more in FEV_1 or until the highest concentration of NKA was reached.

2.6. Determination of CS-003 and metabolites in plasma

For the determination of the level of R-112075, the free base of CS-003 and the metabolites R-112072, R-116274 and R-133740 blood samples were drawn pre-dose as well as immediately after each NKA-provocation test (1,8 and 24h post-dose).

Each sample was drawn (10 mL) into lithium heparin tubes. The samples were immediately centrifuged ($1600g$, 15 min, at 4 °C) and the plasma was stored at -80 °C. The samples were sent on dry ice to MDS Pharma Services (Zürich, Switserland) where they were analysed using validated liquid chromatography/electrospray ionisation-tandem mass spectrometry methods. The lower limit of quantification for all analyses was 0.5ng/mL.

2.7. Statistical analyses

2.7.1. Efficacy parameters

Efficacy was assessed using the provocative concentration of NKA causing a 20% fall of FEV_1. The values were transformed to log_{10} for CS-003 and placebo. The log_{10} values were calculated by linear interpolation based on the last log_{10} concentration of NKA that caused a fall of 20% of FEV_1. For analysing the data ANOVA was used, with factors for patient, period and treatment. If the PC_{20} FEV_1 for NKA was not reached after inhaling the highest concentration, a value of PC_{20} FEV_1 for NKA at $10^{-5.5}$ mol/mL was assigned. Values are reported as mean ± standard deviation (SD). Level of significance was set at $p < 0.05$.

2.7.2. Pharmacokinetic parameters

The relationship between plasma concentrations of the free base of CS-003 (R-l 12075) at 1, 8 and 24h post-dose and the delta log_{10} PC_{20} FEV_1NKA (CS-003 minus placebo) was analysed by Spearman's rank-order correlation coefficient.

3. Results

3.1. Patients

Sixteen patients (9 males, 7 females) were randomised and 15 patients completed the trial as intended. The mean age was 29.8 years ± 5.8 (mean ± SD). Their mean (±SD) baseline FEV_1 was 3.45 ±0.62 L or 90.4 ± 8.0% predicted. Their mean PC_{20} FEV_1 for NKA was $1.09\pm1.00 \times 10^{-7}$ mol/mL at screening (Table 1).

3.2. The effect of CS-003 on baseline FEV_1

There was no statistically significant difference between the baseline 1 (= pre-challenge) and baseline 2 (= post-diluent) mean FEV_1 values on the treatment (CS-003) (3.42 ± 0.62 and 3.34 ± 0.62L) and the placebo day (3.43

± 0.69 and 3.25 ± 0.71 L) at 1 h post-dose.

3.3. Reproducibility of the NKA provocation

At screening, all patients had an NKA-provocation test with a mean $\log_{10}$ PC_{20} FEV_1 for NKA -7.20 ± 0.57 $\log_{10}$-mol/mL. After placebo, the mean $\log_{10}$ PC_{20} FEV_1 for NKA was -6.99 ± 0.66, -6.82 ± 0.37 and -7.12 ± 0.65 at 1, 8 and 24 h, respectively. The maximal fall in percentage at which the PC_{20} was reached was $28.3 \pm 5.4\%$ at screening and $29.3 \pm 11.3\%$, $25.5 \pm 4.6\%$ and $28.1 \pm 9.9\%$ after 1, 8 and 24 h post-dose on the placebo days.

3.4. Effect of CS-003 on the NKA-induced broncho constriction

The mean $\log_{10}$ PC_{20} FEV_1 for NKA (+SD) was -7.20 ± 0.57 $\log_{10}$mol/mL at screening. After intake of CS-003, the mean $\log_{10}$ PC_{20} FEV_1 for NKA was -5.92 ± 0.83 $\log_{10}$ mol/mL at 1 h post-dose and in the placebo group it was -6.99 ± 0.66 $\log_{10}$ mol/mL ($p<0.05$).

Table 1: *Patient characteristics at screening*

Characteristics	
Age (yr)	29.8 ± 5.8
Weight (kg)	70.1 ± 12.1
FEV_1 (L)	3.45 ± 0.62
$FEV_1\%$ predicted	90.4 ± 8.0
PC_{20} for NKA (mol/mL $\times 10^{-7}$)	1.09 ± 1.00
Log_{10} PC_{20} for NKA (mol/mL $\times 10^{-7}$)	-7.20 ± 0.57

Data presented as mean $\pm$ SD. FEV_1: forced expiratory volume in 1 s; PC_{20}: provocative concentration causing a 20% fall in FEV_1; NKA: neurokinin A.

At 8h post-dose, the mean $\log_{10}$ PC_{20} FEV_1 for NKA ($\pm$SD) was -6.39 ± 0.80 $\log_{10}$mol/mL in the CS-003 group compared to $-6.82 \pm 0.37$$\log_{10}$mol/mL for the placebo group ($p<0.05$) (Fig. 1). In 12 out of 16 and 5 out of 16 patients, 1 and 8h after dosing, respectively, a 20% fall in FEV_1 was not reached after inhaling the highest dose of NKA (Fig. 2). For those patients, a value of PC_{20} FEV_1 for NKA at $10^{-5.5}$mol/mL was assigned. After 24 h post-dose, no statistical significant difference was found between the CS-003 and placebo treatment (PC_{20} FEV_1 for NKA -7.24 ± 0.73 $\log_{10}$mol/mL versus PC_{20} FEV_1 for NKA was $-7.12 \pm 0.65$$\log_{10}$mol/mL) (Table 2).

3.5. Plasma concentration of CS-003

After intake of 200 mg CS-003, the mean concentrations varied by factor 3. Plasma concentration of the free base of CS-003 (R-112075) after 1h post-dose was 1196 ± 423ng/mL (mean + SD). After 8h post-dose the concentration was 56 ng/mL, at 24 h the concentration was only measurable in a few patients. All metabolites were less concentrated than the parent drug. All the Spearman's rank-order correlation coefficients were close to zero which suggests that there was no correlation between the plasma concentration of R-l 12075 and the $\log_{10}$ PC_{20} FEV_1NKA values.

3.6. Drug safety

Two adverse events were reported by two patients during treatment with CS-003. One patient had to withdraw from the trial due to an exacerbation of asthma, the other patient complained about moderate nausea. The nausea was stated as definite drug-related. Both adverse events recovered completely within the follow-up period. No severe adverse events were found.

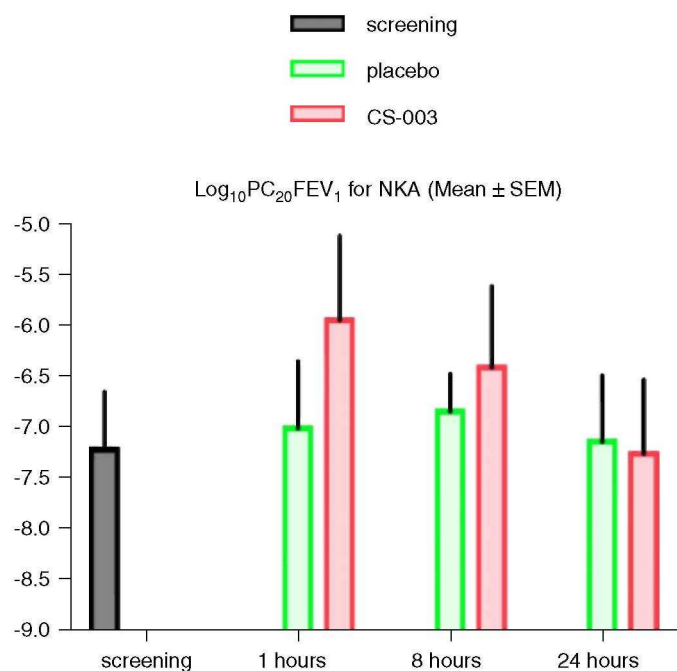**Fig. 1:** *$\log_{10} PC_{20} FEV_1$; for NKA (mean $\pm$ SD) [$\log_{10}$mol/mL].*

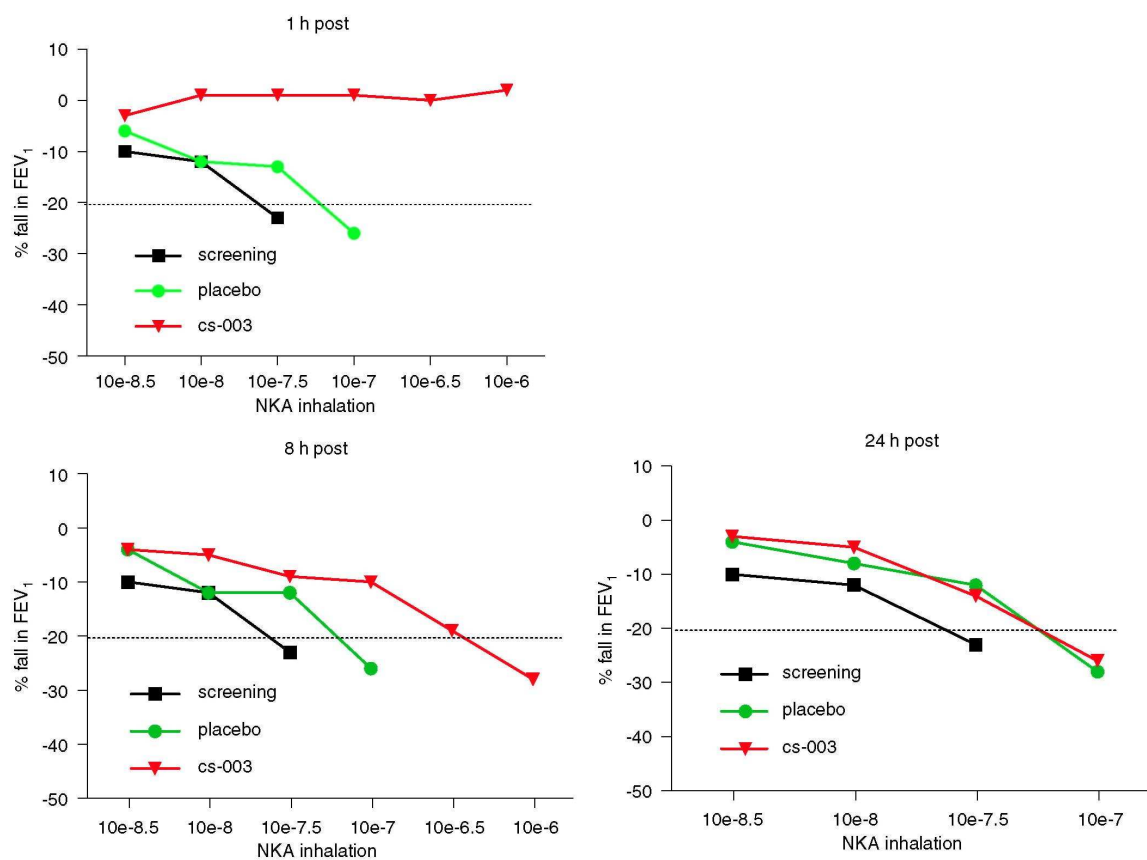**Fig. 2:** *Example of dose-response curve for one patient at 1, 8 and 24 h post-dose.*

Table 2: *$Log_{10}\ PC_{20}\ FEV_1$; NKA-ANOVA and 95% confidence intervals at different time points*

Time post-dose (h)	Mean difference CS-003-placebo [log_{10}mol/mL]	Lower bound (95% conf. interval)	Upper bound (95% conf. interval)
1	1.004	0.511	1.497
8	0.496	0.060	0.933
24	-0.237	-0.679	0.205

4. Discussion

CS-003, a triple tachykinin receptor antagonist, shows high affinities for human NK_1, NK_2 and NK_3 receptors with around subnanomolar K_i values. Comparing K_i value of CS-003 with those of other selective tachykinin receptor antagonists, the affinity of CS-003 was about 10 times weaker than that of FK 888 (a selective NK_1 receptor antagonist) for NK_1 receptor, nearly equal to that of SR 48968 for NK_2 receptor and about 10 times stronger than that of SB 223956 (a selective NK_3 receptor antagonist) for NK_3 receptor [21]. In guinea pigs, intravenously injected (i.v.) CS-003 inhibited capsaicin-induced bronchoconstriction in a dose-dependent manner. CS-003 (1.0mg/kg, i.v.) inhibited the bronchoconstriction over 80%, while SR 48968 (10 mg/kg, i.v.) inhibited it only about 60% and the effects of FK 888 (10mg/kg, i.v.) and SB 223956 (10mg/kg, i.v.) were much weaker than that of SR 48968 [21].

In this trial, we have demonstrated that CS-003 inhibited NKA-induced bronchoconstriction in mild to moderate asthmatic patients. A significant protective effect was observed 1 and 8 h after administration of the drug, while baseline lung function was not affected.

This is the first demonstration of activity of a triple tachykinin receptor antagonist in human airways. There was a definite shift to the right in the dose-response curve for NKA. At 1 h post-dose there was a 10-fold difference in the PC_{20} values between CS-003 and placebo, at 8 h postdose this difference was 3-fold. In comparison with other clinical trials with tachykinin receptor antagonists, the protective effect of CS-003 against NKA-induced bronchoconstriction was larger. This effect is even an underestimation since 12 out of 16 and 5 out of 16 patients at 1 and 8h post-dose, respectively, showed complete protection for NKA even with the highest NKA challenge dose.

Saredutant and nepadutant, both NK_2 receptor antagonists, had a smaller protective effect against NKA [17,18]. DNK333 was the first dual NK_1/NK_2 receptor antagonist which showed a clear inhibition of NKA-induced bronchoconstriction. The protective effect of DNK333 was shown at 1 h post-dose (protection in 15 out of 18 patients) but not at 10h post-dose [19].

We could not show a relationship between increases in plasma concentration and the shift in the dose-response curve to inhaled NKA. Also in the trial with DNK 333, there was no relationship between the plasma drug concentration and the bronchoprotection [19]. This can be explained by a relatively low number of observations (16 patients) and a rather high inter-patient variability in provocative concentration of NKA. We still found a 3-fold protection at 8h post-dose, a time point where all circulating drugs has disappeared. This can be explained by possible accumulation of the drug in the airways, or the binding to the NK_1, NK_2 and NK_3 receptors is long lasting.

The pronounced inhibiting effect of the triple tachykinin receptor antagonist CS-003 on the NKA-induced bronchoconstriction in asthmatic patients raises the question whether all three tachykinin receptors play an important role in the bronchoconstrictor effect of NKA. Although NK_2 receptors play a prominent role in the bronchoconstrictor effect of tachykinins [7], NK_1 receptors can also mediate the bronchoconstriction, especially due to their presence in the small- and medium-sized bronchi. Immune histochemistry has demonstrated the presence of both NK_1 and NK_2 receptors in the smooth muscle of the human lung. Moreover, NK_1 receptors are also located on the blood vessels, inflammatory and neuronal cells, and are mediating the bronchoconstrictor effect of NKA [10,11]. NK_3 receptors have been shown to mediate cough and bronchial hyperresponsiveness, at least in animal models [12]; moreover, they have been implied in the regulation of bronchial parasympathetic ganglion neurotransmission in human airways [13].

Blocking all three receptors results in the best protection up till now against NKA-induced bronchoconstriction. The only way we can prove that this larger effect is caused by blocking the NK_3 receptor is to perform a comparable trial with a selective NK_3-receptor antagonist in combination with NK_1/NK_2 receptor antagonist or matched placebo.

In conclusion, we have demonstrated that CS-003 is able to block NKA-induced bronchoconstriction in patients with asthma. So this is the first triple tachykinin receptor antagonist that has been shown active in human airways. Moreover, this compound posed no major drug-related safety problems in single dosing. So, this compound should be evaluated further in clinical asthma, using lung function, symptom scores and quality of life as clinical endpoints.

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