

The NK-2 receptor antagonist SR 48968C does not improve adenosine hyperresponsiveness and airway obstruction in allergic asthma

J. KRAAN*, H. VINK-KLOOSTER† and D. S. POSTMA*

Departments of *Pulmonology and †Lung Function, University Hospital Groningen, Groningen, The Netherlands

Summary

Background When stimulated, excitatory nonadrenergic noncholinergic (e-NANC) nerves locally release tachykinins like Neurokinin (NK) A and Substance P, causing neurogenic inflammation and airway obstruction via activation of specific NK-1 and NK-2 receptors. The recently developed nonpeptide NK-2 receptor antagonist SR 48968C has a high affinity for the NK-2 receptor, and is a strong and selective antagonist of NK-2 receptor mediated airway obstruction.

Objective and methods In a placebo-controlled cross-over study, we investigated the effect of SR 48968C, administrated orally once-daily in a dosage of 100 mg during 9 days, on airway responsiveness to adenosine 5'-monophosphate (AMP) in 12 allergic asthmatic patients. Furthermore, we assessed its effect on airway obstruction, by measuring FEV₁ on the first and last day of each treatment period and by peak flow registration at home throughout the study period.

Results and conclusion SR 48968C had no significant effect on PC₂₀AMP or on FEV₁ measured on day 1 and 9, and morning and evening peakflow measured at home on day 2–8. Thus, although SR 48968C was administrated in a dosage that might cause a demonstrable blocking effect on airway NK-2 receptors in asthma, it did not have a significant bronchodilatory or bronchoprotective effect against adenosine hyperresponsiveness in this study. Further studies are needed to assess the value of SR 48968C and other NK receptor antagonists in the treatment of asthma

Keywords: asthma, adenosine hyperresponsiveness, neurokinins, NK-2 receptor antagonist, SR 48968C

Clinical and Experimental Allergy, Vol. 31, pp. 274–278. Submitted 27 July 1999; revised 18 February 2000; accepted 30 May 2000.

Introduction

The possible role of excitatory nonadrenergic noncholinergic nerve (e-NANC) pathways in the pathogenesis of airway inflammation and bronchial hyperresponsiveness in asthma has been subject of extensive research in recent years [1]. A dense sensory nerve network, consisting of unmyelinated C-fibres, has been demonstrated to be present subepithelially

in animal and human airways. The tachykinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are expressed, costored and coreleased from these capsaicin-sensitive nerves.

Stimulation of fibre endings via airway epithelium by irritant or chemical triggers such as cigarette smoke, capsaicin, histamine and bradykinin leads to release of these neuropeptides, which exert their effects via interaction with three types of tachykinin receptors, the neurokinin (NK)-1, NK-2 and NK-3 receptors [2]. SP, NKA and NKB have predominant affinity for the NK-1, NK-2, and NK-3

Correspondence: Dr J. Kraan, Department of Pulmonology, University Hospital Groningen, Postbox 30.001, 9700 RB Groningen, The Netherlands. E-mail: j.kraan@int.azg.nl

receptors, respectively, but all tachykinins can act as full agonist for these receptors. Activation of NK-1 receptors mediates effects like vasodilation, plasma extravasation, inflammatory cell recruitment, and airway mucus secretion. These effects are known by the term neurogenic inflammation. Activation of NK-2 receptors by NKA results in contraction of airway smooth muscle.

In normal and asthmatic subjects, SP and NKA are present in bronchoalveolar lavage (BAL) fluid [3–4]. In some studies increased amounts of SP have been demonstrated in BAL fluid and sputum of asthmatic subjects [3,4]. In one study, however, NKA was shown to be the predominant tachykinin in human BAL fluid [5]. Furthermore, increased expression of NK-1 and NK-2 receptor mRNA in bronchial tissue has been demonstrated in asthmatic subjects [6–7]. Asthmatics are more sensitive to the bronchoconstrictive effect of NKA than healthy individuals [8]. They also show an increased bronchial responsiveness to histamine 24 h after inhalation of SP [9]. Thus, by their presence and ability to elicit relevant pathophysiological effects in the airways, tachykinins fulfil two important criteria as possible mediators in asthma.

The development of potent and highly selective tachykinin receptor antagonists has made it possible to further examine the contribution of e-NANC nerve pathways to the pathophysiology of asthma. These agents may constitute a potential new class of anti-asthma drugs [10].

The recently developed nonpeptide NK-2 receptor antagonist SR 48968C has a high affinity for the NK-2 receptor, and has been shown to be a strong and selective antagonist of NK-2 receptor mediated bronchoconstriction [11]. A single dose of this drug has been shown to effectively inhibit neurokinin-A-induced bronchoconstriction in asthmatics [12].

In a placebo-controlled cross-over study we investigated the effect of SR 48968C, after once-daily oral administration of 100 mg, for 9 days. We did not only investigate its bronchodilator effect, but also its bronchoprotective effect on the indirect bronchoconstrictor stimulus adenosine 5'-monophosphate (AMP). Several studies have demonstrated that AMP hyperresponsiveness is as useful a marker of disease activity in asthma as histamine or methacholine hyperresponsiveness [13]. AMP acts as a bronchoconstrictor via histamine release from mast cells, and there is also evidence that AMP may cause bronchoconstriction via stimulation of tachykinin releasing sensory nerves [14]. Thus the NK-2 receptor antagonist SR 48968C might inhibit AMP-induced bronchoconstriction by blocking the effect of NKA released from these nerves. We assessed the effect of SR 48968 on bronchial obstruction in these patients by measuring FEV₁ on the first and last day of each treatment period and by peak flow registration at home throughout the study period.

Table 1 Patients characteristics

Number	12
Age (range), years	30 (19–47)
Height (range), cm	183 (175–197)
Weight (range), kg	82 (65–100)
Baseline FEV ₁ (SEM), % pred.	80 (2)
– Reversibility (%)*	20 (2)
PC ₂₀ methacholine (range), mg/mL	1.86 (0.06–2.9)
Medication before study	
– Inhaled β_2 -agonist if needed (<i>n</i>)	11
– Inhaled corticosteroids (<i>n</i>)†	6
– Cromoglycate (<i>n</i>)	2

* Change in FEV₁ in percentage from baseline after inhalation of salbutamol 800 µg via dose-inhaler with spacer.

† Inhaled corticosteroids were withdrawn 3 months before the start of the study.

Methods

Subjects (Table 1)

Twelve non-smoking allergic asthmatic patients gave their informed consent to participate in the study. Male subjects, 18–50 years, were selected on a history of episodic wheezing, consistent with the diagnosis of asthma with no other concomitant illness, house-dust mite allergy as documented by elevated specific IgE (RAST $\geq$ 2 PRU/mL), baseline FEV₁ $\geq$ 60% and $\leq$ 90% of the predicted value [15], an increase in FEV₁ of at least 15% after 800 µg of inhaled salbutamol, the presence of bronchial hyperresponsiveness (BHR), defined by a methacholine PC₂₀ $\leq$ 8 mg/mL. Their weight had to be within 20% of ideal body weight. Short courses of oral corticosteroids had not been used within 2 months, inhaled corticosteroids not within 3 months, and cromoglycate, methylxanthines, long-acting beta-2-agonists or antihistamines not within 2 weeks prior to the study. Throughout the study no drugs other than the study drugs were allowed except inhaled short-acting beta-2-agonists or ipratropiumbromide on an 'if needed' basis. The study was approved by the hospital ethics committee.

Pulmonary function and inhalation provocation test

Slow inspiratory vital capacity (VC) and FEV₁ were measured with a water-sealed spirometer (Lode, the Netherlands). Airway responsiveness was assessed by a methacholine bromide (PC₂₀Mch) or adenosine 5'-monophosphate (AMP) provocation test [16]. The challenge was started with a saline inhalation followed by inhalations of doubling concentrations of MCh bromide (0.038–19.6 mg/mL) or AMP (0.04–640 mg/mL), at 5-min intervals. Single

FEV₁ values were measured 30 and 90 s after each inhalation of MCh or AMP. The inhalations were stopped as soon as the fall in FEV₁ was 20% from baseline FEV₁ or when the highest inhalation concentration was reached.

Study design

The study was performed using a randomized, double blind, placebo-controlled, cross-over design. Patients were randomly allocated in order to constitute two sequences of either placebo-SR 48968C (six patients) or SR 48968C-placebo (six patients). The two study periods were separated by a washout period of 1–3 weeks. Patients visited the clinic on study days 1 and 9 of each treatment period, and stayed overnight at the clinic from day 9 to day 10. Capsules of SR 48968C (four capsules of 25 mg each) or placebo (four capsules) were taken orally at 08.00 on study days 1, 9 and 10, and at 07.00–09.00 on study days 2–8, after overnight fasting. The use of an inhaled short-acting beta-2-agonist or ipratropiumbromide was allowed during the study, except for the measurement days in the hospital, from 8 h prior to the first lung function test. No other treatment was allowed during the whole study period. The patients did not drink caffeine-containing beverages on the hospital study days.

On study days 1 and 9 FEV₁ measurements were made at 1 h before and each 10' during the first hour, and consequently every hour until 10 h after study drug intake. On study day 9 FEV₁ was measured at 11 h, 12 h, 13 h, 14 h, 20 h and 24 h post-dose. Finally, on day 10 an AMP challenge was performed 1 h after taking the study drug. On days 2–8 patients measured morning (between 07.00 and 09.00) peak flow (Mini-Wright) before and 30 min after drug intake and in the evening at 20.00.

Statistical analysis

Logarithmic transformation of the AMP PC₂₀ was carried out before statistical analysis. The sequential FEV₁ values for each measurement day were transformed to a single area under the curve value. Comparison between the effect parameters of the two treatment periods was carried out using ANOVA. This includes testing on sequence effects, and residual effects of the first treatment on the second treatment. Data are expressed in mean $\pm$ SEM.

Results

Spirometry and peak flow measurements at home

Baseline FEV₁ values (% of predicted $\pm$ SEM) in the two treatment sequence groups (SR 48968C-placebo or vice versa) were 76 ± 5 and 81 ± 5 , respectively (n.s.). There

Table 2 Effect of SR 48968C vs. placebo on FEV₁

FEV ₁ , % predicted	SR 48968C		Placebo	
	Day 1	Day 9	Day 1	Day 9
Baseline	77 ± 4	77 ± 5	80 ± 3	76 ± 4
30 min	79 ± 5	78 ± 4	79 ± 3	77 ± 4
1 h	78 ± 4	75 ± 4	78 ± 4	77 ± 4
6 h	81 ± 4	80 ± 4	80 ± 4	80 ± 3
10 h	77 ± 4	81 ± 4	77 ± 3	76 ± 4
20 h		77 ± 5		76 ± 4
24 h		81 ± 4		78 ± 3

FEV₁ values are expressed as percentage predicted ($\pm$ SEM).

was a small but significant difference in AUC-FEV₁ values measured on day 9 between the two treatment sequences. This effect was caused by a small increase in FEV₁ on day 9 in both treatment periods in the patients who were in sequence placebo-SR, as opposed to a trend towards decrease on this day in the patients in sequence SR-placebo. No such effect was observed on treatment day 1. There was no significant residual effect of either of the treatments.

Effects of SR 48968C, and placebo, on FEV₁ (% of predicted), measured after the first dosage (day 1) and after 8 days of treatment (day 9) are shown in Table 2. The peak flow measurements, recorded at home on days 2 and 8, are shown in Table 3. There were no significant treatment effects on FEV₁, expressed as AUC-FEV₁, neither was there a significant effect on peak flow values during the whole treatment period.

Effect on PC₂₀-AMP

The effects of SR 48968C and placebo on PC₂₀-AMP, as measured at day 9 of each treatment period, are presented in Table 4. Individual values and the geometric mean values are presented. PC₂₀-AMP could not be obtained in

Table 3 Effect of SR 48968C vs. placebo on peak flow measured at home

Peak flow, % predicted	SR 48968C		Placebo	
	Day 2	Day 8	Day 2	Day 8
Morning				
– Baseline	83 ± 5	89 ± 3	83 ± 4	84 ± 4
– 30' after drug	86 ± 4	89 ± 4	86 ± 4	88 ± 4
Evening	93 ± 3	94 ± 3	91 ± 3	92 ± 3

Peak flow values are expressed as percentage predicted ($\pm$ SEM).

Table 4 Effect of nine days treatment with SR 48968C vs. placebo on PC₂₀-AMP

Patient	SR 48968C PC ₂₀ AMP,mg/mL	Placebo PC ₂₀ AMP,mg/mL
1	0.02	0.75
2	9.49	5.37
3	6.10	0.47
4	0.02	0.03
5	5.89	2.93
6	2.95	1.39
7	0.60	0.34
8	44.2	27.8
9	101	67.4
10	1.29	2.12
11	18.3	14.5
12	6.47	6.02
Geometric mean	2.75	2.37
Mean ² log (PC ₂₀)	1.46 (1.11)*	1.25 (0.98)*

*SEM

two subjects, because FEV₁ already decreased by 20% after the control saline inhalation at the start of AMP provocation. In these cases, we used for statistical purposes the lowest AMP inhalation provocation concentration (0.02 mg/mL). We found no significant treatment effect on the PC₂₀-AMP.

Discussion

In the present study we investigated the effect of the nonpeptide NK-2 receptor antagonist SR 48968C on bronchial obstruction in stable allergic asthmatic patients. Because SR 48968C might also offer protection against bronchoconstrictory stimuli (especially stimuli caused via a neuronal reflex mechanism) while not having a clear bronchodilating effect, we also studied the effect of this drug on bronchial hyperresponsiveness, as assessed with AMP provocations.

We selected AMP as a bronchoconstrictor agent because it has been shown that AMP hyperresponsiveness is a useful marker of disease activity in asthma. In addition there is evidence that activation of neural pathways and release of tachykinins by these nerves might contribute to the bronchoconstriction induced by AMP [14].

We did not find any improvement in lung function nor in AMP-responsiveness after 9 days of oral treatment with SR 48968C, 100 mg orally once daily. It is known from *in-vitro* studies that SR 48968C has a high affinity for NK-2 receptors in various species including man [11,17]. It potentially antagonizes NK-2 receptor mediated bronchoconstriction of isolated guinea pig and human airway smooth

muscle [11]. SR 48968C produces a dose-dependent inhibition of bronchoconstriction induced by NKA in anaesthetized guinea pigs [18]. It also has been found to have an inhibitory effect on the NK-2 receptor-mediated component of bronchoconstriction caused by inhaled citric acid [19], capsaicin [20] and ovalbumine (in ovalbumine-sensitized animals [21]). In a recent study in allergic asthmatic subjects by van Schoor *et al.* an oral dose of 100 mg SR 48968C protected significantly against bronchoconstriction induced by inhaled NKA [12].

Thus the oral dosage of 100 mg SR 48968C is sufficient to deliver a significant blockade of airway smooth muscle NK-2 receptors in man. Consequently, one might presume that SR 48968C, when administered in this way, is an appropriate pharmacological agent to investigate the NK-2 receptor mediated component of the bronchial obstruction and BHR in allergic asthma. The lack of efficacy of SR 48968C in our study can therefore not easily be explained by the dose of the drug, although it might still be possible that higher dosages or longer treatment duration might elicit bronchodilatory or bronchoprotective effects.

There is abundant evidence to consider a possible role of tachykinins in the pathophysiology of asthma and bronchial hyperresponsiveness. NKA has been demonstrated in bronchoalveolar fluid of normal and asthmatic subjects, and in one study it appeared to be the predominant tachykinin in BAL fluid [5]. In another study increased amounts of SP-immunoreactivity were demonstrated in BAL fluid and induced sputum of asthmatics when compared to nonasthmatic subjects, with a further rise after allergen challenge [3]. In asthmatic subjects, increased expression of NK-1 and NK-2 receptor mRNA in bronchial tissue has been demonstrated [6–7]. Asthmatics are more sensitive to the bronchoconstrictive effect of NKA than healthy individuals [8, 22]. Furthermore, asthmatics show an increased bronchial responsiveness to histamine 24 h after inhalation of SP [9].

In animal studies NK receptor antagonists have been demonstrated to prevent allergen and toluene diisocyanate-induced bronchial hyperresponsiveness. SR 48968C, as a selective NK-2 receptor antagonist, was effective in preventing the allergen induced bronchial hyperresponsiveness in guinea pigs [21], whereas the NK-1 specific receptor antagonist RP 67580 was effective in mice [23]. These results underline the possibility that species differences exist in the contribution of the different types of NK receptors in NANC nerve pathways.

It follows from the evidence accumulated in the above mentioned previous research, that even when treatment of asthma patients with SR 48968C in a higher dosage or for a longer treatment duration would not result in improvement of AMP responsiveness or FEV₁, still, additional investigations are necessary to gain more insight into the role of

NK-receptor mediated pathways in the pathophysiology of asthma. For example, the effect of NK-receptor blocking agents on other parameters such as allergen-induced hyper-responsiveness, and subjective asthma symptoms such as chest tightness and cough, should be studied. Furthermore, from the above noted species differences that appear to be present in the contribution of the various types of NK receptors in the NANC nerves, it may be concluded that studies with combined NK-1 and NK-2, or even NK-1, NK-2 and NK-3, receptor antagonist have to be carried out to demonstrate or rule out the possible role of NANC nerves in asthma.

In conclusion, SR 48968C, when given orally for 9 days in allergic asthmatic patients, did not have a significant bronchodilatory effect or a bronchoprotective effect against adenosine hyperresponsiveness in this study. It is clear that further research is necessary to obtain more insight into the role of tachykinins in asthma and the possible place of NK receptor antagonists in the treatment of this disease.

Acknowledgements

This study was supported by Sanofi, France.

References

- Joos GF, Germonpre PR, Pauwels RA. Neurogenic inflammation in human airways: is it important? *Thorax* 1995; 50:217–9.
- Advenier C, Joos GF, Molimard M et al. Role of tachykinins as contractile agonists of human airways in asthma. *Clin Exp Allergy* 1999; 29:579–84.
- Nieber K, Baumgarten CR, Rathack R et al. Substance P and β -endorphin-like immunoreactivity in lavage fluid of subjects with and without allergic asthma. *J Allergy Clin Immunol* 1992; 60:646–52.
- Tomaki M, Ichinose M, Miura M et al. Elevated substance P content in induced sputum from patients with asthma and patients with chronic bronchitis. *Am J Respir Crit Care Med* 1995; 151:613–7.
- Heaney LG, Cross LJM, McGarvey LPA et al. Neurokinin A is the predominant tachykinin in human bronchoalveolar fluid in normal and asthmatic subjects. *Thorax* 1998; 53:357–62.
- Adcock IM, Peters M, Gelder C et al. Increased tachykinin receptor gene expression in asthmatic lung and its modulation by steroids. *J Mol Endocrinol* 1993; 11:1–7.
- Bai TR, Zhou D, Weir T et al. Substance P (NK₁) and neurokinin A (NK₂)-receptor gene expression in inflammatory airway diseases. *Am J Physiol* 1995; 269:L309–17.
- Joos G, Pauwels R, Van Der Straeten M. The effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects. *Thorax* 1987; 42:779–83.
- Cheung D, van der Veen H, den Hartigh J et al. Effects of inhaled substance P on airway responsiveness to metacholine in asthmatic subjects in vivo. *J Appl Physiol* 1994; 77:1325–32.
- Advenier C, Lagente V, Boichot E. The role of tachykinin receptor antagonists in the prevention of bronchial hyper-responsiveness, airway inflammation and cough. *Eur Respir J* 1997; 10:1892–906.
- Emonds-Alt X, Vilain P, Goulaouic P et al. A potent and selective non-peptide antagonist of the neurokinin A (NK₂) receptor. *Life Sci* 1992; 50:PL101–6.
- Van Schoor J, Joos G, Chasson BL et al. The effect of the NK₂ tachykinin receptor antagonist SR 48968 (saredutant) on neurokinin A-induced bronchoconstriction in asthma. *Eur Resp J* 1998; 12:17–23.
- Polosa R, Holgate ST. Adenosine bronchoprovocation: a promising marker of allergic inflammation in asthma? *Thorax* 1997; 52:919–23.
- Polosa R, Rajakulasingam K, Church MK et al. Repeated inhalation of bradykinin attenuates adenosine 5'-monophosphate (AMP) induced bronchoconstriction in asthmatic airways. *Eur Respir J* 1992; 5:700–6.
- Quanjer PhH, ed. Standardized lung function testing. *Clin Resp Physiol*; 1983 19(Suppl. 5):45–51.
- Oosterhoff Y, Koëter GH, de Monchy JGR et al. Circadian variation in airway responsiveness to methacholine, propranolol, and AMP in atopic asthmatic subjects. *Am Rev Respir Dis* 1993; 147:S12–S17.
- Gether U, Yokota Y, Emonds-Alt X et al. Two non-peptide tachykinin antagonists act through epitopes on corresponding segments of the NK₁ and NK₂ receptors. *Proc Natl Acad Sci USA* 1993; 90:6194–8.
- Floch A, Fardin V, Cavero I. Characterization of NK₁ and NK₂ tachykinin receptors in guinea-pig and rat bronchopulmonary and vascular systems. *Br J Pharmacol* 1994; 111:759–68.
- Satoh H, Lou YP, Lundberg JM. Inhibitory effects of capsazepine and SR 48968 on citric acid-induced bronchoconstriction in guinea-pigs. *Eur J Pharmacol* 1993; 236:367–72.
- Lou YP, Lee LY, Satoh H et al. Postjunctional inhibitory effect of the NK₂ receptor antagonist SR 48968, on sensory NANC bronchoconstriction in the guinea-pig. *Br J Pharmacol* 1993; 109:765–73.
- Bertrand C, Geppetti P, Graf PD et al. Involvement of neurogenic inflammation in antigen-induced bronchoconstriction in guinea pigs. *Am J Physiol* 1993; 265:L507–11.
- Cheung D, Timmers MC, Zwinderman AH et al. Neutral endopeptidase activity and airway hyperresponsiveness to neurokinin A in asthmatic subjects in vivo. *Am Rev Respir Dis* 1993; 148:1467–73.
- Scheerens H, Buckley TL, Muis T et al. The involvement of sensory neuropeptides in toluene diisocyanate-induced tracheal hyperactivity in the mouse airways. *Br J Pharmacol* 1996; 119:1665–71.