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Short communication

Inhibition of inflammatory cell recruitment by the tachykinin NK_3-receptor antagonist, SR 142801, in a murine model of asthma

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Abstract

Several observations suggest that tachykinins (substance P, neurokinin A and neurokinin B) are involved in the pathogenesis of pulmonary diseases and elicit several airway responses such as bronchoconstriction and neurogenic inflammation via interactions with specific receptors denoted NK_1, NK_2 and NK_3. We have investigated the effect of a selective antagonist for tachykinin NK_3 receptor, SR 142801 ((*R*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl-*N*-methylacetamide), on the inflammatory cell recruitment in ovalbumin-sensitized and -challenged mice used as a model of allergic asthma. Twenty hours after the two-ovalbumin challenges, differential cell counts were calculated and indicated that SR 142801 caused a significant decrease in the number of neutrophils and eosinophils. Forty hours after the last ovalbumin exposure, SR 142801 induced a significant decrease in the recruitment of eosinophils. These results suggest that tachykinins and tachykinin NK_3 receptors can interfere with cell recruitment in inflammatory response.

Keywords: Tachykinin NK_3 receptor; Ovalbumin; Inflammatory cell; Bronchoalveolar lavage; (Mouse); Tachykinin

1. Introduction

Tachykinins are a group of neuropeptides including substance P, neurokinin A and neurokinin B. These mediators are released from capsaicin sensitive sensory C-fibres upon nerve stimulation and have been shown to elicit several airway responses such as bronchoconstriction and "neurogenic inflammation". Neurogenic inflammation includes microvascular leakage, vasodilatation, mucus secretion and recruitment and activation of inflammatory cells. The biological actions of tachykinins are mediated by three types of receptors denoted NK_1, NK_2 and NK_3 which have the highest affinity for substance P, neurokinin A and neurokinin B, respectively (for review, see Regoli et al., 1994). It has been demonstrated that bronchoalveolar lavage fluid and induced sputum from asthmatics contain increased amounts of substance P like immunoreactivity and that substance P and neurokinin A contract human airways in vitro and in vivo; neurokinin A being more potent than substance P and asthmatics being more sensitive than normal subjects (for review, see Spina and Page, 1996; Joos et al., 2000). Progress in investigations on the physiological and pathological roles of tachykinins has been greatly facilitated by the availability of a number of highly selective non-peptide antagonists for tachykinin NK_1, NK_2 and NK_3 receptors. Tachykinin NK_2 receptors have been shown to be involved in bronchoconstriction and bronchial hyperresponsiveness whereas tachykinin NK_1 receptors were found to be mainly involved in neurogenic inflammation. For example, we have demonstrated the importance of tachykinin NK_2 receptor stimulation in the development of antigen-induced airway hyperreactivity in the guinea pigs with the highly selective tachykinin NK_1 and NK_2 receptor antagonist, SR 140333 [(*S*)1-(2-(3-(3,4-dichlorophenyl)-1-(3-isopro-poxyphenylacetyl)piperidine-3-yl)ethyl)-4-phenyl-1-azoniabicyclo (2.2.2) octane,chloride] and SR 48968 [(*S*)-*N*-methyl-*N*(4-acetylamino-4-phenyl-piperidino-2-(3,4-dichlorophenyl) butyl)-

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benzamide], respectively (Boichot et al., 1995). However, the involvement of tachykinin NK_1 and NK_2 receptors in inflammatory cell recruitment is debated (Lagente and Advenier, 1998; Kraneveld et al., 2000). Indeed, using the same model, we have reported that SR 140333 and SR 48968, administered intraperitoneally, did not reduce cell recruitment in bronchoalveolar lavage fluid (Boichot et al., 1998). In contrast, Schuiling et al. (1999a,b) have shown that the aerosol administration of SR 140333 in guinea pigs was able to reduce the allergen-induced infiltration of eosinophils, neutrophils and lymphocytes and that SR 48968 could decrease the allergen-induced infiltration of neutrophils and lymphocytes in the airways.

The role played by tachykinin NK_3 receptors in the control of bronchial reactivity remains undetermined but the discovery of the non-peptide antagonist of this receptor, SR 142801 ((*R*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl-*N*-methylacétamide), has opened new possibilities in this area. It has been recently reported that SR 142801 was able to inhibit cough, bronchial hyperresponsiveness, and exaggerated plasma extravasation in response to histamine induced by the administration of substance P or citric acid to guinea pigs (Daoui et al., 1997, 1998) suggesting a role in lung pathology.

In order to investigate the involvement of tachykinin NK_3 in airway inflammation, we analyzed the effects of inhaled SR 142801 on the inflammatory cell recruitment in sensitized and challenged mice used as an experimental model of allergic asthma.

2. Materials and methods

2.1. Animals and experimental protocols

Ten-week-old male balb/c mice (CERJ, Le Genest Saint Isle, France) were sensitized by an i.p. injection of 0.5 ml solution of ovalbumin (180 μg/ml) (chicken egg grade V, Sigma, Saint Louis, USA) adsorbed with aluminium hydroxide (4 mg/ml) on days 0 and 7. On day 18, mice were exposed to an aerosol of ovalbumin in 0.9% NaCl (5 mg/ml for 30 min and then 10 mg/ml for 30 min), twice 4 h apart. Mice exposed to an aerosol of saline constituted control group. Aerosols were generated by a SPAG-2 nebulisator (ICN Pharmaceuticals, Costa-Mesa, CA, USA). This system should nebulize the drug solution from the reservoir at a rate of 12.5–15 ml/h and produce particle size with an average of 0.3–0.5 μm.

2.2. Treatment of mice with SR 142801

One hour before each ovalbumin challenge, sensitized mice were treated by the tachykinin NK_3 receptor antagonist, SR 142801 (Sanofi-Recherche, Montpellier, France). This compound was dissolved in ethanol and diluted with saline to obtain the appropriate concentration. Then, it was administrated by aerosol for 30 min at a 10^{-6} M dose using the SPAG-2 nebulisator. Control mice sensitized to ovalbumin but exposed to saline were exposed to SR 142801. As another control, some mice sensitized and exposed to ovalbumin received saline instead of SR 142801 by aerosol for 30 min, 1 h before the antigen challenge.

2.3. Bronchoalveolar lavage

After the last challenge (20 or 40 h), bronchoalveolar lavage were performed. Mice were anaesthetized i.p. with pentobarbital sodium (120 mg/kg) (Sanofi, Santé Animale, France). After semi excision of the trachea, a plastic canula was inserted and airspace was washed with 0.5 ml of 0.9% NaCl containing 2.6 mM EDTA with a 1-ml syringe. This operation was realized 10 times. Bronchoalveolar lavages were centrifuged ($600 \times g$ for 10 min, 4°C). After lysis of erythrocytes with distilled water, cell pellets were suspended in 500 μl of 0.9% NaCl containing 2.6 mM EDTA. Total cell count was performed using a hemacytometer chamber and cell viability was determined by the trypan blue exclusion method. Cytospins were then realized at 1000 rpm for 2 min (CYTOPRO 7620-Wescor) and cells were stained using the May-Grünwald Giemsa method. Differential cell counts were calculated on 200 cells using standard morphological criteria.

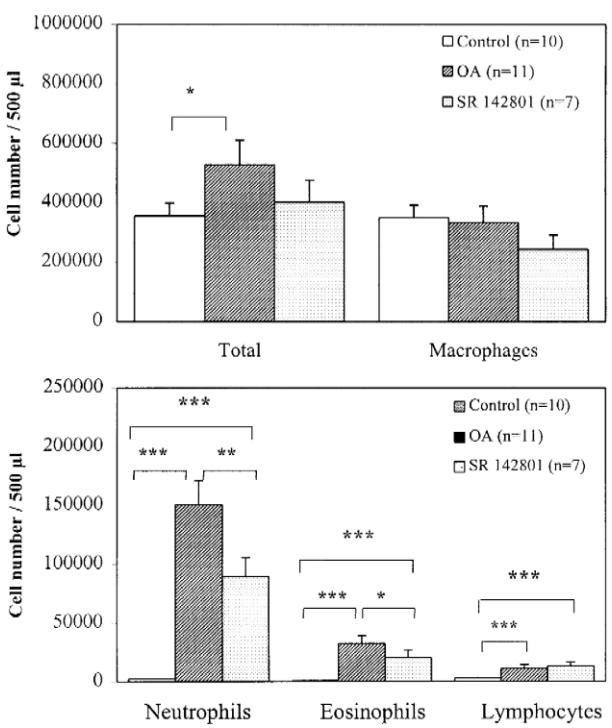

Fig. 1. Effect of SR 142801 treatment (10^{-6} M, aerosol exposure for 30 min) on the total and differential cell numbers in bronchoalveolar lavages recovered 20 h after the last ovalbumin exposure in sensitized mice. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

2.4. Statistical analysis

Results were expressed as means ± S.E.M. Groups were compared using analysis of variance (ANOVA) and the Student's *t*-test. For each analysis, P values less than 0.05 were considered as statistically significant.

3. Results

3.1. Cellular changes in bronchoalveolar lavage fluids after ovalbumin exposure

Exposure to ovalbumin induced a significant increase ($P < 0.05$) in the total cell number when bronchoalveolar lavages were realized 20 h after the last challenge (Fig. 1A). However, 40 h after the last ovalbumin exposure, the total cell number was not significantly modified in comparison with control mice (Fig. 2A).

Exposure to ovalbumin challenge led to a diversification of cellular composition in bronchoalveolar lavage fluids. Twenty hours after the last ovalbumin exposure, the number of macrophages was not modified in comparison with control mice but there was an important increase ($P < 0.001$) in the number of neutrophils (Fig. 1A and B). Eosinophils and lymphocytes were also significantly increased ($P < 0.001$) but in a restrained way (Fig. 1B). Forty hours after the last challenge, the number of macrophages was not modified (Fig. 2A) but there was a significant and important increase ($P < 0.001$) in the number of eosinophils and lymphocytes (Fig. 2B). The neutrophil number was also increased but in a restrained way ($P < 0.001$) (Fig. 2B).

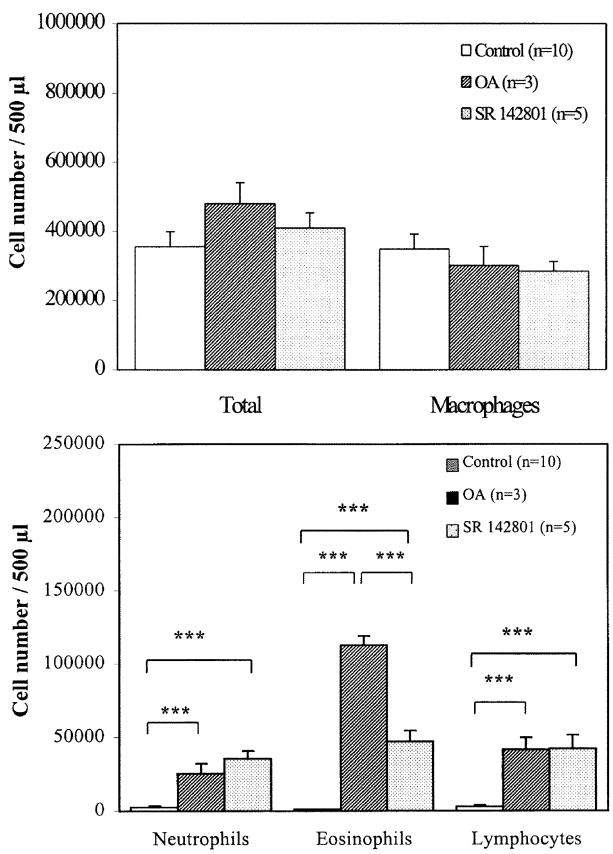

Fig. 2. Effect of SR 142801 treatment (10^{-6} M, aerosol exposure for 30 min) on the total and differential cell numbers in bronchoalveolar lavages recovered 40 h after the last ovalbumin exposure in sensitized mice. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

3.2. Effect of SR 142801 on inflammatory cell recruitment

When bronchoalveolar lavages were realized 20 h after the last challenge, treatment by SR 142801 at 10^{-6} M (30 min, 1 h before each challenge) induced a significant decrease ($P < 0.01$) in the number of neutrophils. A significant reduction in eosinophil number was also observed ($P < 0.05$). No effect on the number of lymphocytes was noted (Fig. 1B).

Treatment by SR 142801 at 10^{-6} M induced a marked and significant decrease ($P < 0.001$) in the number of eosinophils when bronchoalveolar lavages were performed 40 h after the ovalbumin exposure. No effect on the number of neutrophils and lymphocytes was observed (Fig. 2B).

4. Discussion

The present study demonstrates for the first time that an aerosol exposure of the tachykinin NK_3 receptor antagonist, SR 142801, significantly reduces the inflammatory cell recruitment in airways induced by ovalbumin challenge in sensitized mice.

Previous studies have already shown that the very potent and selective tachykinin NK_3 receptor antagonist SR 142801 was able to inhibit cough, bronchial hyperresponsiveness and exaggerated plasma extravasation response to histamine induced by substance P or citric acid in guinea pig (Daoui et al., 1997, 1998), that is one of the main characteristics of inflammation observed in asthmatics. Another characteristic of this process is the local inflammatory cell recruitment.

First of all, we have presently reported that the aerosol exposure of Balb/c mice to ovalbumin induced a recruitment of inflammatory cells in bronchoalveolar lavage fluids. Interestingly, there is a differential accumulation of leukocytes subtypes in bronchoalveolar lavage fluids consisting of an early recruitment neutrophils 20 h after the last antigen exposure and then, a late increase of eosinophils and lymphocytes 40 h after the last ovalbumin challenge. These data suggest two phases of the inflammatory process: an early neutrophilic response and a later lymphocyte/eosinophil response as previously demonstrated in mice (Lukacs et al., 1994), guinea pigs (Boichot et al.,

1991) and in human asthma (Metzger et al., 1986). The kinetics of the passage of the neutrophils and the eosinophils through the airway walls probably explains the late appearance of the latter type in the bronchoalveolar lavage fluids, even though marked differences between the alveolar space (as reflected by the cell composition of the bronchoalveolar lavage fluids) and the lung tissue (as assessed by histological techniques) may exist (Boichot et al., 1991).

To investigate the involvement of tachykinin NK_3 receptors in inflammatory cell recruitment, a solution of SR 142801 (10^{-6} M) was administered by aerosol before each antigen challenge. Treatment of mice with SR 142801 significantly reduced the influx of neutrophils and eosinophils 20 h after the last ovalbumin challenge and the influx of eosinophils 40 h after. These results confirm the potential implication of tachykinin NK_3 receptor in airway inflammatory response and more generally the implication of neuropeptides like tachykinins. However, the role of tachykinin in the modulation of leukocyte chemotaxis is debated (Lagente and Advenier, 1998). Indeed, we have shown in sensitized guinea pigs that ovalbumin aerosol induced an increase in the total number of cells and granulocytes in bronchoalveolar lavage fluids that was not reduced by pre-treatment of animals with a single dose of SR 140333 or SR 48968 (1 mg/kg) administrated intraperitonally (Boichot et al., 1998). But, it has been recently established that the tachykinin NK_2 receptor antagonist, SR 48968, administered by aerosol at the concentration of 10^{-7} M to sensitized and exposed to ovalbumin guinea pigs significantly reduced neutrophil and lymphocyte influx 25 h after the last ovalbumin exposure but remained ineffective on eosinophil influx (Schuiling et al., 1999a). Using the same model of allergic asthma, it was also demonstrated that SR 140333, NK_1 receptor antagonist, used at the same concentration, could significantly inhibit eosinophil, neutrophil and lymphocyte influx (Schuiling et al., 1999b).

We have investigated the effect of the tachykinin NK_3 receptor antagonist, SR 142801, administered by aerosol at the concentration of 10^{-7} M. As this treatment remained ineffective in our model (data not shown), we have used the concentration of 10^{-6} M that permitted to reduce inflammatory cell recruitment. Recently, Sarau et al. (2001) reported that there are specie differences in the potencies of tachykinin receptor antagonists in murine and human NK_3 receptor with a lower potencies in the former. These data would be explain the absence of results obtained with 10^{-7} M SR 142801 in contrast to that it was observed using 10^{-7} M SR 140333 and SR 48968 in the guinea pig (Schuiling et al., 1999a,b).

However, generally, it seems that tachykinin NK_1 and NK_2 receptors and their preferential ligands, respectively, substance P and neurokinin A, are involved in inflammatory cell accumulation in lung but the involvement of tachykinin NK_3 receptor has never been demonstrated until now. Nevertheless, blockade of both tachykinin NK_1 and NK_3 receptor attenuated the leukocyte trafficking induced by bradykinin B_1 receptor activation and substance P in mice (Mc Lean et al., 2000).

In the present state of our knowledge, it is difficult to clarify the mechanism and/or the site of action of SR 142801. First of all, the action of this substance cannot be ascribed to an antagonistic activity at tachykinin NK_1 and/or NK_2 receptors. Indeed, in vivo and in vitro experiments have clearly demonstrated that SR 142801 is a potent and selective antagonist of the tachykinin NK_3 receptors (Emonds-Alt et al., 1995). Moreover, on the basis of Myers and Undem's (1993) results, it has been recently proposed that the effects of SR 142801 could be linked to the inhibition of tachykinin NK_3 receptor localized on bronchial parasympathetic ganglion neurons (Daoui et al., 2000). With regard to the effect of SR 142801 on inflammatory cell recruitment, the mechanism of action is more complex to explain. However, interestingly, it has been shown that co-cultured B- and T-lymphocytes could express tachykinin NK_3 receptor mRNA only after activation by interleukin-5 and transforming growth factor$_{\beta 2}$ (Braun et al., 1999). Hence, we can hypothesize that the effects observed with SR 142801 in this study could be linked to a similar mechanism involving the induction of the expression of tachykinin NK_3 receptor on target cells.

In conclusion, the present study shows that the aerosol administration of SR 142801 is able to reduce inflammatory cell recruitment. These data suggest the involvement of tachykinin NK_3 receptor in this process that has never been observed until now. However, the precise mechanism of action has to be determined.

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