

Neurokinin-1 Receptor Antagonist Inhibits Short-Term Sulfuric-Acid-Induced Airway Hyperresponsiveness in Sensitized Guinea Pigs

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Key Words

Sulfuric acid · Airway hyperresponsiveness · NK₁ receptor · NK₂ receptor

Abstract

Background: Tachykinins are involved in the development of bronchial inflammation and airway hyperresponsiveness (AHR); however, the role of the neurokinin-1 (NK₁) receptor in acid-aerosol-induced bronchial impairment in asthmatic patients remains controversial. **Methods:** To investigate the effects on the NK₁ receptor antagonist FK888 the neurokinin-2 (NK₂) receptor antagonist SR48968 on sulfuric-acid (H₂SO₄)-induced AHR in guinea pigs, specific airways resistance (sRaw) and airways responsiveness to methacholine (MCh) were measured before and after 6 h of exposure to H₂SO₄ aerosol (pH 1.7, 82 mg/m³) in ovalbumin-sensitized guinea pigs. **Results:** Airway responsiveness to MCh significantly increased ($p < 0.05$) after the exposure, however sRaw did not. Treatment with FK888 significantly inhibited ($p < 0.05$) H₂SO₄-induced AHR in a dose-dependent manner, as did SR48968. **Conclusions:** These results suggest that not only NK₂ but also NK₁ receptors might have important roles in the development of acid-aerosol-induced AHR.

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Introduction

Acid fog is a complex mixture of atmospheric pollutants containing multiple stimuli to human airways. We have previously reported that hospital visits for asthma increased on foggy days [1], and an epidemiological study showed that fog was the greatest contributing factor to asthma-related visits compared to other meteorological factors and air pollutants [2]. Acid fog is associated with air pollutants, including nitrogen oxides and sulfur dioxides (SO₂), and most fog acidity is attributable to sulfuric acid (H₂SO₄) and nitric acid. Of these air pollutants, H₂SO₄ appears to be the greatest irritant [3] and has been shown to induce bronchoconstriction and airway hyperresponsiveness (AHR) in human and animal experiments [3–5].

Tachykinins have been shown to have an important role in AHR and bronchoconstriction [6–12]. In exposure experiments with guinea pigs, it was reported that the selective tachykinin neurokinin-2 (NK₂) antagonist but not the selective neurokinin-1 (NK₁) antagonist inhibited SO₂-induced bronchoconstriction [8], and that NK₂ antagonists inhibited citric-acid-induced AHR whereas NK₁ antagonists did not [10]. In addition, NK₂ but not NK₁ antagonists have been shown to reduce allergen-induced AHR at 24 and 48 h after allergen challenge [6]. NK₂ receptor antagonists prevented AHR more clearly than NK₁ receptor antagonists [6, 7, 10, 11], suggesting that NK₂ receptor stimulation plays an important role in the development of AHR. However, the effects of NK₁ receptor antagonists remain controversial [12].

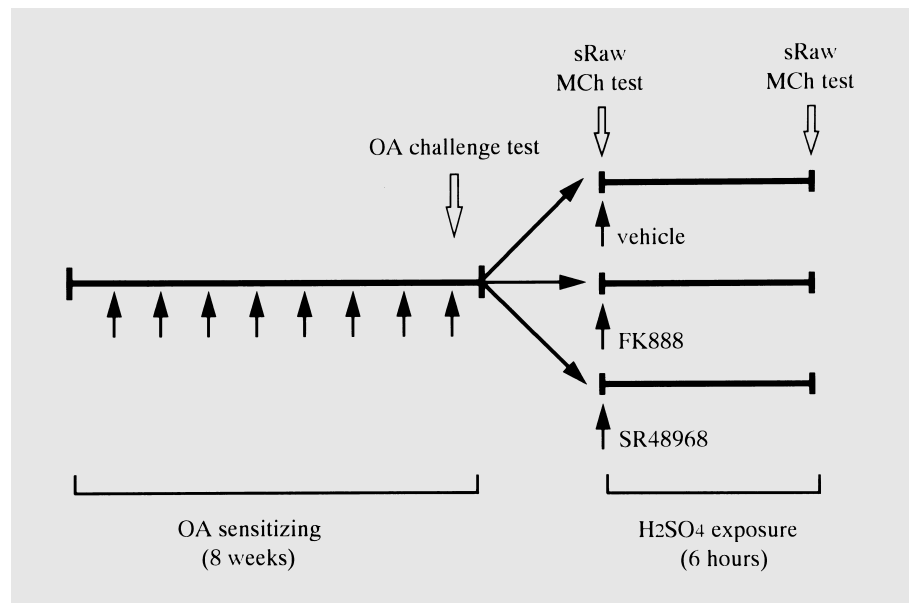


Fig. 1. Protocol of experiments.

The purpose of this study was to examine whether NK₁ receptor mechanisms are involved in H₂SO₄-induced AHR and airway resistance. We examined the effects of selective NK₁ and NK₂ receptor antagonists on ovalbumin (OA)-sensitized guinea pigs following inhalation of H₂SO₄.

Materials and Methods

Animals and Drugs

Male Hartley guinea pigs weighing 250–300 g (Nihon SLC, Shizuoka, Japan) were used and were given water and commercial food ad libitum. The NK₁ receptor antagonist FK888 was supplied by Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan and the NK₂ receptor antagonist SR48968 by Sanofi Recherche, Montpellier, France.

Study Design

As shown in figure 1, all guinea pigs were sensitized by aerosolized 1% OA generated with an ultrasonic nebulizer (NE-U10B; Omron, Toyo, Japan) and the sensitization was performed for 10 min, once a week for 8 weeks. Then the OA challenge test was given and an early asthmatic reaction was observed in all animals. The OA-sensitized guinea pigs were then divided into three treatment groups: the vehicle group [dimethyl sulfoxide (DMSO), subcutaneously], the NK₁ receptor antagonist group (FK888 dissolved in DMSO at a dose of 0.2, 1.0 and 2.0 mg/kg body weight, subcutaneously) and the NK₂ receptor antagonist group (SR48968 dissolved in saline at a dose of 1.0, 2.0 and 4.0 mg/kg body weight, intraperitoneally). These pretreatments were performed 30 min before H₂SO₄ aerosol exposure. Each group contained 5 guinea pigs.

All animals inhaled H₂SO₄ aerosol for 6 h in a polyacrylamide box. The aerosol was generated by the nebulizer from 0.02 N H₂SO₄ solution (pH = 1.7, 40 mosm). The concentration of the H₂SO₄ in the box was measured by ion chromatography (model 2010i; Dionex, Sunnyvale, Calif., USA), and was maintained at 82±4 mg/m³.

Measurement of Airway Function

Specific airway resistance (sRaw) and airway responsiveness to methacholine (MCh) were measured before and after 6 h of exposure to H₂SO₄. sRaw was measured by the detection of respective sensors of air flow attached to the front and rear chambers according to the method described by Pennock et al. [13]. Briefly, an awake guinea pig was positioned with its neck extending through the partition of a two-chambered, whole-body plethysmograph. Each chamber was fitted with identical wire screen pneumotachographs and identical differential pressure transducers (model DP-45; Validyne Corp., Northridge, Calif., USA). These transducers were connected to a respiratory analyzer (Pulmos-I; MIPS, Osaka, Japan), and a personal computer was used for the on-line breath-by-breath measurement of sRaw.

Airway responsiveness was evaluated by airway response to MCh. MCh aerosols were generated by an ultrasonic nebulizer (model T-10; Sinei Industries Inc., Saitama, Japan) with a flow rate of 0.8 liters/min delivered into the chamber. MCh solutions were prepared in concentrations of 0.0078, 0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/ml. After the animals had inhaled saline and each MCh aerosol for 1 min, sRaw was measured for 1 min. Inhalation was repeated with aerosols of increasing concentrations until sRaw increased to more than twice the baseline value, which was defined as sRaw after saline aerosol inhalation. Airway responsiveness to MCh was evaluated by the concentration of MCh required to increase sRaw to twice the baseline value (PC200-MCh).

Statistical Analysis

The changes in sRaw were analyzed using Wilcoxon's test. The changes in PC200-MCh by administration of FK888, SR48968 or vehicle were calculated using logarithmic values, defined as Δlog PC200-MCh and analyzed by analysis of variance and Fisher's PLSD test. All results are expressed as the mean ± SD. p values < 0.05 were considered significant.

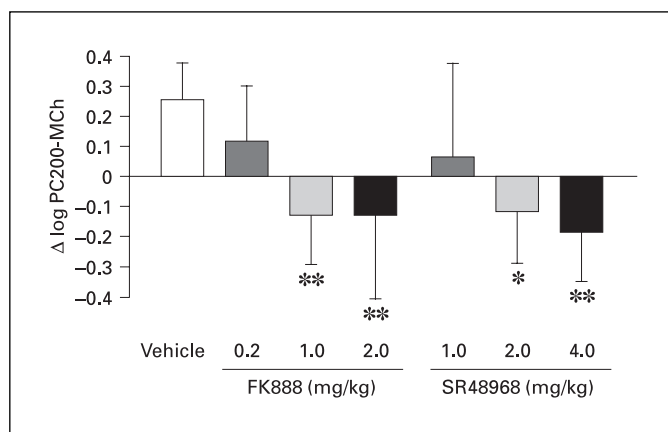


Fig. 2. Effects of FK888 and SR48968 on H₂SO₄-induced AHR to inhaled MCh in guinea pigs (n = 5 in each groups). Pretreatment by FK888 and SR48968 inhibited AHR in a dose-dependent manner. Mean ± SD. *p<0.05, **p<0.01 compared with vehicle.

Results

sRaw did not significantly increase after 6 h of exposure to H₂SO₄ aerosol in the vehicle group. The sRaws of the FK888 and SR48968 pretreatment groups also remained unchanged by the exposure.

As shown in figure 2, ΔlogPC200-MCh in the vehicle group was 0.24±0.14. The ΔlogPC200-MCh of the FK888 pretreatment group at 0.2, 1.0 and 2.0 mg/kg was 0.12±0.17, -0.12±0.17 and -0.12±0.27, respectively, and pretreatment with 1.0 and 2.0 mg/kg of FK888 significantly inhibited (p<0.01) H₂SO₄-induced AHR to MCh compared with the vehicle group. The ΔlogPC200-MCh of the SR48968 pretreatment group at 1.0, 2.0 and 4.0 mg/kg was 0.06±0.33, -0.12±0.17 and -0.18±0.17, respectively, and a dose of 2.0 and 4.0 mg/kg significantly inhibited AHR (p<0.05; p<0.01, respectively).

Discussion

We found that not only the NK₂ but also the NK₁ receptor antagonist inhibited H₂SO₄-induced AHR in OA-sensitized guinea pigs, suggesting that the NK₁ receptor might play an important role in the development of AHR by inhalation of an acid aerosol. Tachykinins such as substance P (SP) and neurokinin A (NKA) are localized in unmyelinated C-fiber afferent endings in the airways, and NK₁ and NK₂ receptors have the highest affinity for SP and NKA, respectively [14]. Additionally, NK₁ receptors appear to be main-

ly involved in airway microvascular leakage, vasodilation, mucus secretion and inflammatory cell responses, whereas NK₂ receptors play a role in bronchoconstriction [12, 15]. In guinea pigs, it has been reported that the selective NK₂ receptor antagonist SR48968, but not the selective NK₁ receptor antagonist CP99994, inhibited SO₂-induced bronchoconstriction [8], and that SR48968 inhibited citric-acid-induced AHR whereas the NK₁ antagonist SR140333 did not [10]. Also, NK₂ but not NK₁ antagonists have been shown to reduce allergen-induced AHR at 24 and 48 h after allergen challenge [6]. Boichot et al. [16] showed that NK₁ antagonists inhibited the increase of microvascular leakage of histamine in SP-exposed guinea pigs, but did not prevent the development of AHR, and suggested a lack of association between the presence of microvascular leakage and the occurrence of AHR. Advenier et al. [12] suggested that NK₁ mediated the development of microvascular leakage hypersensitivity, while NK₂ mediated the development of AHR by exposure to SP or citric acid in guinea pigs, and that NK₂ receptor stimulation plays an important role in the development of AHR, while the effects of NK₁ receptor antagonists are still being debated.

By contrast, Schuiling et al. [17] recently reported that the selective NK₁ receptor antagonist SR140333 reduced OA-induced AHR to histamine after both the early and late asthmatic reactions in guinea pigs. Our data are in agreement with these results. Inflammations mediated by the NK₁ receptor induce airway vasodilation, mucus secretion, inflammatory cell responses and microvascular leakage. We hypothesized that H₂SO₄-induced AHR might partly be promoted by the stimulation of airway C-fiber evoked by the direct and/or indirect effects of H₂SO₄-associated injury of epithelial cells, and that consequently the tachykinin SP might induce microvascular leakage hypersensitivity. This hypothesis is in agreement with a previous finding that NK₁ but not NK₂ antagonists inhibited airway microvascular leakage induced by inhaled sodium metabisulfite in guinea pigs [18].

Previous studies have been undertaken with concentrations of H₂SO₄ aerosol ranging from 0.1 to 72.7 mg/m³ [3, 19–21], but we used a high concentration of H₂SO₄ aerosol, 82 mg/m³, because we were attempting to induce AHR in a short exposure. However, in our study, the changes in sRaw before and after 6 h of exposure to H₂SO₄ aerosol were not significant. Silbaugh et al. [21] reported individual differences in responsiveness to H₂SO₄ aerosol in each guinea pig. Indeed, in 2 of our 5 guinea pigs, sRaw obviously increased after exposure, but in the other 3 it did not. On the other hand, AHR was enhanced by H₂SO₄ inhalation in 4 of the guinea pigs, so it seems that the development of airway

bronchoconstriction and responsiveness do not always correspond in guinea pigs.

In conclusion, our results showed that both a selective NK₁ and NK₂ receptor antagonist could inhibit H₂SO₄-induced AHR in sensitized guinea pigs. Our results also sug-

gest that neuropeptides might contribute to the development of AHR induced by H₂SO₄. Further investigations into NK₁ and NK₂ receptor antagonists will be useful to establish their viability in clinical application for asthmatic patients whose condition is aggravated by acid fog.

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