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Effect of TA-270, a novel quinolinone derivative, on antigen-induced nasal blockage in a guinea pig model of allergic rhinitis

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ABSTRACT

TA-270 (4-hydroxy-1-methyl-3-octyloxy-7-sinapinoylamino-2(1H)-quinolinone) is a novel quinolinone derivative that has been demonstrated to possess an anti-oxidative activity against peroxynitrite, a potent oxidant, that is generated by the reaction of nitric oxide with superoxide anions. The current study describes the inhibitory effect of TA-270 on the biphasic nasal blockage induced by repeated antigen challenge in an allergic rhinitis guinea pig model. In the present in vitro study, TA-270 potently inhibited the oxidative reaction induced by peroxynitrite (IC_{50}=79 nM). In addition, TA-270 (0.3–30 mg/kg, p.o.) dose-dependently inhibited peroxynitrite (3 mM, 10 μl/nostril)-induced nasal blockage in guinea pigs. In the antigen-induced allergic rhinitis model, TA-270 (0.3, 3, and 30 mg/kg, p.o.) given 1 h before the antigen challenge suppressed early phase nasal blockage by 36%, 42%, and 63%, respectively. Furthermore, TA-270 (0.3, 3, and 30 mg/kg, p.o.) showed a relatively strong suppression of late phase nasal blockage (39%, 62%, and 72%, respectively). The late phase nasal blockage was significantly inhibited (61%) even when TA-270 (30 mg/kg, p.o.) was administered 18 h before the antigen challenge. In conclusion, TA-270 improved antigen-induced nasal blockage, probably through its peroxynitrite scavenging action, and the effect was sustained for at least 18 h. Thus, TA-270 would be expected to relieve nasal blockage in allergic rhinitis patients.

1. Introduction

Patients with allergic rhinitis have serum IgE antibody against specific allergens included in pollens of trees, grasses or weeds, and their characteristic symptoms of the condition are nasal blockage, sneezing, and rhinorrhea (Naclerio, 1991). Nasal blockage is considered to be the most serious problem for patients suffering from allergic rhinitis. When a specific allergen is applied to the nasal cavities of allergic rhinitis patients, over 90% show an immediate nasal blockage response, sneezing and rhinorrhea. In addition, approximately 50% develop a late phase reaction, with the predominant symptom of nasal blockage (Ilipoulos et al., 1990; Pelikan 1978). The nasal mucosa contains venous sinusoids, and an increase in blood flow into the sinusoids produces a rapid reduction in the volume of the nasal airway. Thus, it is strongly suggested that nasal blockage is mainly induced by dilation of the blood vessels in the sinusoids (Eccles, 1995).

Nitric oxide (NO) is known as a powerful vasodilator that modulates systemic vascular tone (Rees et al., 1989). We have demonstrated that a non-selective NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), strongly suppresses both early and late phase nasal blockages (Imai et al., 2001). In addition to its direct vasodilatative action, NO rapidly reacts with superoxide anions, which are released from inflammatory cells such as neutrophils (Salman-Tabcheh et al., 1995; McCall et al., 1989; Carreras et al., 1994; Sutherland et al., 1993; Weiss, 1989), macrophages (Avron and Gallily, 1995; Rodenas et al., 1995), eosinophils (Pincus et al., 1982; Nabe et al., 1998a; Hashimoto et al., 2003) and endothelial cells (Szabo et al., 1995; Kooy and Poyall, 1994), and this results in the formation of peroxynitrite, a highly proinflammatory molecule. It has been reported that the levels of nitrotyrosine that are formed after peroxynitrite attacks tyrosine residues are markedly elevated in the nasal mucosa of patients with perennial allergic rhinitis, but are absent in nonallergic patients (Sato et al., 1998). Furthermore, we reported that the intranasal instillation of peroxynitrite induced nasal blockage in guinea pigs (Mizutani et al., 2008). Taken together, these findings suggest that peroxynitrite may be a novel target in the development of new drugs for allergic rhinitis.

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TA-270 (4-hydroxy-1-methyl-3-octyloxy-7-sinapinoylamino-2 (1H)-quinolinone), a novel quinolinone derivative, was designed as an antioxidant to scavenge reactive oxygen species. TA-270 has been demonstrated to possess an anti-oxidative activity against the potent oxidant peroxynitrite. Furthermore, it has been reported that TA-270 improved biphasic asthmatic responses and hyperresponsiveness in a guinea pig model of allergic asthma, and its inhibitory effect was markedly stronger than that of a cysteinyl leukotriene antagonist (Aoki et al., 2000). Based on these results, TA-270 is currently being developed for clinical use as an anti-asthmatic drug.

In the present study, in order to assess the potential value of TA-270 as a therapeutic agent for allergic rhinitis, we evaluated the effect of TA-270 on allergic biphasic nasal blockage in an experimental allergic rhinitis model.

2. Material and method

2.1. Animals

Male std: Hartley guinea pigs (3–5 weeks of age) were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed under controlled temperature (24±1 °C) and humidity (55±5%) and were given access to food and water ad libitum.

All of the experimental procedures were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.2. Reagents

TA-270 and pranlukast were synthesized by DIC Corporation (Chiba, Japan). Japanese cedar pollen (*Cryptomeria japonica*) was harvested in Gifu and Shiga prefectures in 1998. Peroxynitrite and 3-(4-morpholinyl) sydnonimine hydrochloride (SIN-1) were obtained from Dojindo Laboratory (Kumamoto, Japan).

Peroxynitrite (3 mM) was diluted in phosphate buffered saline (PBS) 1 min before the intranasal instillation. For the negative control, decomposed peroxynitrite was prepared by leaving the solution at room temperature for more than 1 month in sterile conditions.

$Al(OH)_3$ gel was prepared using 0.25 N NaOH and 0.25 N $Al_2(SO_4)_3$, as described previously (Nabe et al., 1997a).

The cedar pollen extract used for sensitization was prepared as described previously (Nabe et al., 1998b). Briefly, pollen was suspended in PBS at 100 mg/ml and kept at 4 °C for 18 h with mild stirring. The suspension was then centrifuged (1700 ×g, 15 min), and the supernatant was stored at −80 °C until it was used as the sensitization antigen.

2.3. Scavenging effect of TA-270 on peroxynitrite-mediated dihydrorhodamine (DHR)-123 oxidation

The scavenging effect of TA-270 on peroxynitrite-mediated DHR-123 oxidation was assessed as follows: TA-270 (6.3–800 nM) was added to 0.1 M phosphate buffer solution (pH 7.4) containing 5 μM DHR-123 solution in a final volume of 2994 μL. The mixture was subjected to fluorescence measurement (excitation wavelength/absorption wavelength=500 nm/536 nm). Six μl of 5 mM SIN-1 solution (a peroxynitrite generator) were then added 4 min after the measurement, and the fluorescence was measured for 8 min. Changes in the rate of oxidation in each group were calculated based on the change before and after adding SIN-1.

2.4. Measurement of nasal blockage

sRaw was measured by a two-chambered, double-flow plethysmograph system in accordance with the method of Pennock et al. (1979). In brief, an animal was placed with its neck extending through the partition of a two-chambered box, and after airflow detection using sensors attached to both the front and rear chambers, sRaw was measured with a Data analyzer Pulmos-I (M.I.P.S., Osaka). The change in sRaw was expressed as the % increase from the pre-challenge baseline value (before).

2.5. Effect of TA-270 on peroxynitrite-induced nasal blockage

To induce sRaw elevation by peroxynitrite, peroxynitrite (3 mM, 10 μl/nostril) was intranasally instilled into normal guinea pigs as reported previously (Mizutani et al., 2008). sRaw was measured 10 min after the instillation. It has been reported that instillation of peroxynitrite causes a swift elevation of sRaw that peaks at 10 min and then decreases at 20 min.

TA-270 (0.3–30 mg/kg) or pranlukast (100 mg/kg) was orally administered 1 h before the peroxynitrite instillation.

2.6. Sensitization and challenge with cedar pollen

The animals were sensitized with Japanese cedar pollen according to the method described by Nabe et al. (1998b). Briefly, the animals were sensitized by intranasal instillation of cedar pollen extracts adsorbed onto $Al(OH)_3$ gel at a concentration of 0.3 μg protein/0.3 mg $Al(OH)_3$/3 μl/nostril twice daily for 7 days. Then, the sensitized animals were bilaterally intranasally challenged once a week by inhalation of cedar pollen using a hand-made inhalation apparatus, which allowed quantitative inhalation of pollen at a dose of 1.8 mg/nostril (Nabe et al., 1997b).

2.7. Effect of TA-270 on the biphasic nasal blockage induced by pollen inhalation challenge

TA-270 (0.3, 3 and 30 mg/kg) or pranlukast (30 mg/kg) was orally administered 1 h before the 6th antigen challenge in this allergic rhinitis model. Furthermore, in order to investigate whether the effect of TA-270 on the biphasic nasal blockage was sustained after an oral administration, TA-270 (30 mg/kg) was administered 1, 18, or 24 h before the 10th antigen challenge.

2.8. Effect of TA-270 on the nasal blockage induced by leukotriene D_4 (LTD_4) instillation

Nasal responsiveness tests described previously (Mizutani et al., 2001) were performed according to the following procedure: Two doses of LTD_4 (10^{-8} and 10^{-6} M, 10 μl/nostril) were consecutively instilled at a 20-min interval into the bilateral nostrils of the sensitized guinea pigs 2 days after the 15th antigen challenge. sRaw was measured 10 min after the respective instillations. TA-270 (30 mg/kg) was orally administered 1 h before the LTD_4 (10^{-8} M) instillation.

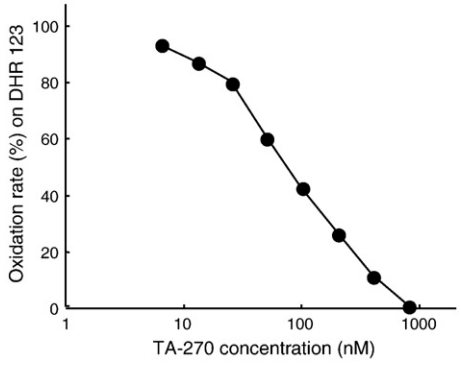

Fig. 1. Effect of TA-270 on the oxidation of DHR123 by the peroxynitrite generator SIN-1. DHR: dihydrorhodamine, SIN-1: 3-(4-Morpholinyl) sydnonimine hydrochloride.