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The effect of a cysteinyl leukotriene antagonist, ONO-1078 (pranlukast) on agonist- and antigen-induced nasal microvascular leakage in guinea pigs*

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SUMMARY

The in vivo model of nasal microvascular leakage was used for the nasal allergic challenge in ovalbumin (OA)-sensitised guinea pigs, or nasal stimulation with leukotriene D_4 (LTD_4) in non-sensitised animals. An intravenous injection of Evans blue dye was given as an index of nasal microvascular leakage. Following the nasal stimulation with LTD_4, the concentration of dye in the nasal lavage fluid rapidly increased. Oral administration of ONO-1078 (pranlukast) (3-30 mg/kg) significantly inhibited the LTD_4-induced nasal microvascular leakage. In OA-sensitised guinea pigs, the excretions of dye into nasal lavage fluid were recognised soon after the topical antigenic stimulation and continued for over 60 minutes. Oral administration of ONO-1078 (30 mg/kg) significantly inhibited the antigen-induced microvascular leakage. These results suggest that ONO-1078 may be of therapeutic use for nasal allergy.

Key words: ONO-1078, leukotriene antagonist, guinea pig, nasal allergy.

INTRODUCTION

The allergic response is a complex process involving the interaction of many mediators. It is well known that a nasal challenge with histamine causes sneezing, rhinorrhea and nasal mucosal swelling, which are the major symptoms of allergic rhinitis. Similarly, a nasal challenge with cysteinyl (Cys) LT increases nasal blood flow and nasal mucosal swelling (Bisgaard et al., 1984). Furthermore, Cys LT is recovered from the nasal lavage fluid of allergic rhinitis patients after allergen provocation (Shaw et al., 1985; Kojima et al., 1991). These reports suggest that not only histamine but also Cys LT may play an important role in the pathogenesis of allergic rhinitis.

ONO-1078 (pranlukast), 4-oxo-8-[4-(phenylbutoxy) benzoylamino]-2-(tetrazol-5-yl)-4H-l-benzopyran hemihydrate, is a novel compound shown to selectively antagonise LTC_4, - D_4 and E_4 induced bronchoconstriction in guinea pig and human bronchial tissues (Obata et al., 1992; Yamaguchi et al., 1992). In addition, in clinical studies, ONO-1078 is a potent and orally active peptide leukotriene antagonist that inhibits LTD_4- and allergen-induced bronchoconstriction in asthmatic patients (Nakagawa et al., 1990; Taniguchi et al., 1993). We hypothesised that treatment with an LTD_4-receptor antagonist might also benefit patients with allergic rhinitis by modifying their response to allergens.

In the present study, we examined the effect of ONO-1078 on antigen-induced nasal microvascular leakage as assessed by the extravasation of Evans blue dye into the nasal lavage fluid in guinea pig models of allergic rhinitis.

MATERIALS AND METHODS

Method of Sensitisation

Male Dunkin-Hartley guinea pigs weighing 200-250 g were used for the experiments. They were kept in a temperature controlled environment with standard laboratory food and water freely available. A sensitisation with OA was performed as described before (Shirasaki et al., 1992). Briefly, after general sensitisation by intraperitoneal injection of OA (10 ug/kg) and aluminium hydroxide (5 mg/kg) three times at 2-week intervals, they were exposed to an aerosol of 0.1% OA for 1 minute daily during one month. The sensitised animals were studied 2 days after final OA inhalation. The serum antibody titre after the sensitisation was 8 16 times in 8-day homologous PCA.

Method of nasal perfusion

The method we previously reported (Shirasaki et al., 1992) was used for the nasal antigenic challenge in OA-sensitised guinea pigs, and nasal stimulation with LTD_4 in OA-non-sensitised animals. Essentially, under general anaesthesia with pentobarbital

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sodium (30 mg/kg, i.p.), a tracheotomy was performed and a dwelling tube (l mm in diameter), connected to a perfusion pump, was inserted into the choana via the tracheostoma. We used Evans blue dye (20 mg/kg) as an indicator of the nasal exudative reaction. Immediately after the intravenous injection of the dye, the nasal cavity was perfused with warmed saline from the indwelling tube. After having perfused the nasal cavity with saline for 20 minutes, OA (l mg/ml) or LTD_4 dissolved in saline was perfused for 3 minutes. Saline was subsequently perfused for 90 minutes. Each perfusion was performed at the rate of 0.25 ml/min. In a separate series, ONO 1078 or its vehicle (carboxymethylcellulose sodium, CMC) (0.1 ml/kg) was orally administered 30 minutes before the Evans blue dye injection. The nasal lavage fluid dropping from the nostril was collected every 10 minutes in a plastic tube The recovery of the nasal lavage fluid was nearly 100% at each time point. The amount of dye was measured spectrophotometrically (620 nm).

Drugs

The following drugs were used: carboxymethylcellulose sodium (CMC-Na, Wako Junyaku Co., Osaka, Japan), Evans blue dye (Sigma, St Louis, MO), ovalbumin (Seikagaku Co., Tokyo, Japan). ONO-1078 and LTD_4 were synthesised by Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

Statistical analysis

Data are presented as mean ± SE. Statistical analysis was performed by using paired or unpaired students' tests (two-tailed) and a p value of less than 0.05 was considered significant.

RESULTS

Instillation of LTD_4 in the nose caused a dose-dependent increase in Evans blue dye extravasation in the nose (Fig. 1). The inhibitory effect of ONO-1078 was initially determined on LTD_4 1 μM-induced microvascular leakage in the nose. Pre-treatment with ONO-1078 significantly inhibited LTD_4-induced microvascular leakage (Fig. 2). Following antigenic challenge (Fig. 3), the concentration of dye in the nasal lavage fluid of five control animals rapidly increased (pre-treatment: 1.02 ± 0.21; 0-10 min: 8.1 ± 1.4 μg/ml, $p<0.05$). Dye concentrations then remained for over 60 minutes at levels which were significantly higher than prechallenge levels. Pre-treatment with ONO-1078 (30 mg/kg, p.o) tended to reduced the dye concentrations (0-10 minutes: vehicle 8.1 ± 1.1 μg/ml versus ONO-1078 4.3 ± 2.4 μg/ml), and statistically significant inhibition in dye concentration by ONO-1078 was found from between 50 to 70 minutes after the antigen challenge (50-60 minutes: vehicle 5.2 ± 1.11 μg/ml versus ONO-1078 1.8 ± 0.3 μg/ml, $p<0.05$; 60-70 minutes: vehicle 4.3 ± 1.1 μg/ml versus ONO-1078 1.6 ± 0.3 μg/ml, $p<0.05$). The total amount of dye in the nasal lavage fluid from 0 to 60 minutes after the antigen challenge was significantly reduced by ONO 1078 (0-60 min: vehicle 114.4 ± 19.8 μg versus ONO-1078 58.5 ± 22.1 μg/ml, $p<0.05$). The inhibition percentage of the whole response by ONO-1078 was calculated to be 44.7%.

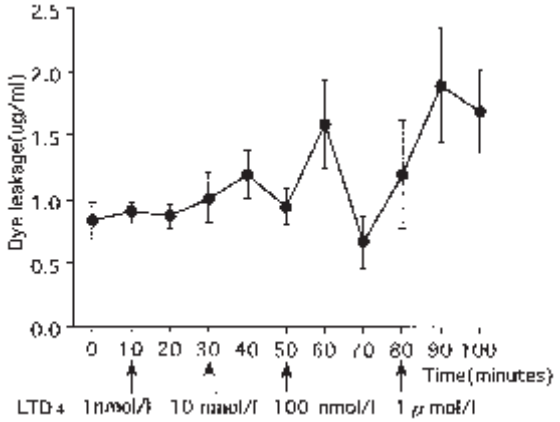

Figure 1. Dye leakage response to topical LTD_4 application. Each point and bar represent mean and SEM of 3 animals.

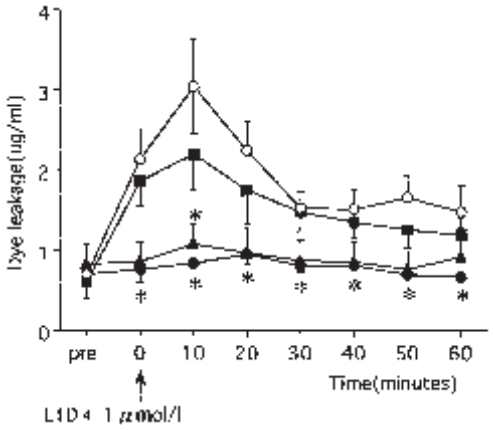

Figure 2. Effect of ONO-1078 on LTD_4 (10^{-6}M)-induced dye leakage response after oral administration of CMC(O), ONO-1078 0.3 mg/kg (■), 3 mg/kg (▲), or ONO-1078 30 mg/kg (●). Each point and bar represent mean and SEM of 5 animals. Significant difference from vehicle (CMC)-treated group is indicated, *p <0.05.

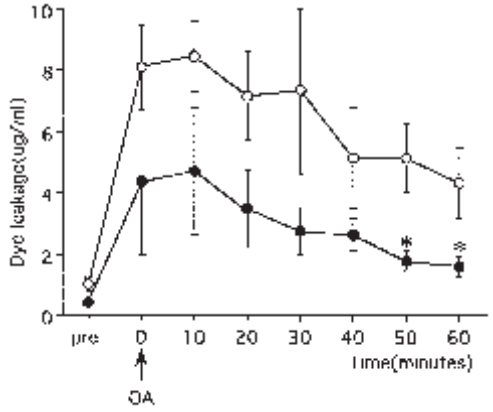

Figure 3. Effect of ONO-1078 on antigen-induced dye leakage response after oral administration of CMC(O), or ONO-1078 30 mg/kg (●). Each point and bar represent mean and SEM of 5 animals. Significant difference from vehicle (CMC)-treated group is indicated, *p<0.05.

DISCUSSION

It has been reported that topical application of LTC_4 causes significant increases of nasal microvascular leakage (Shirasaki et al., 1992) in guinea pigs. In the present study, we found that the LT receptor antagonist, ONO-1078 (3-30 mg/kg, p.o.) inhibited exogenous LTD_4-induced nasal microvascular leakage (Fig.2). With regard to the effect of ONO-1078 on guinea pig airways, it has been demonstrated that ONO-1078 (pranlukast) (0.3-3 mg/kg, p.o.) (Nakagawa et al., 1992) or SB205312 (pranlukast) (0.1 -1 mg/kg, i.v.) (Bochnowics et al., 1995) causes a dose-dependent reduction of LTD_4-induced airway microvascular leakage in the lung. ONO-1078 has been reported as a highly potent, selective and competitive antagonist of peptide leukotrienes that acts with higher affinity at LTD_4 and LTE_4 receptors than at LTC_4 receptors, and ONO-1078 showed no antagonism against histamine and acetylcholine on guinea pig lung either in vitro or in vivo (Obata et al., 1992). Taking these facts into consideration, our data suggest the existence of an LTD_4 receptor in the guinea pig nose. Also, in the present study, we noted that ONO-1078 inhibited antigen-induced nasal microvascular leakage (Fig. 3). In this study, we used OA-sensitised guinea pigs as a model of human nasal allergy. In the same experimental model, we previously noted that:

1) sneezing, discharges and scratching movements (Narita et al., 1992); 2) release of histamine, kinins and leukotriene C_4 (Shirasaki et al., 1992) and PAF (Shirasaki et al., 1990) into nasal lavage fluid; 3) increased nasal vascular permeability (Shirasaki et al., 1992); and 4) increased eosinophil infiltration into nasal mucosa (Shirasaki et al., 1990) occurred following nasal antigen challenge. We noted that NK1 receptor antagonists strongly inhibited the immediate phase of the antigen-induced nasal microvascular leakage in OA-sensitised guinea pigs (Shirasaki et al., 1997). Contrary to this, in this study, LT receptor antagonist ONO-1078 inhibited especially the later phase of the antigen-induced nasal microvascular leakage. We previously observed a prolonged release of LTC_4 into the nasal lavage fluid in OA-sensitised guinea pigs (Shirasaki et al., 1992). From these observations, it was deduced that, in contrast to substance P, LTs might be involved in the prolonged increase of vascular permeability found in this model of nasal allergy. Using the same animal model as the present study, ONO-1078 (3-30 mg/kg, p.o.) inhibited airway resistance after nasal antigen challenge without causing the nasal symptoms (sneezing and scratching) (Narita et al., 1997). In the lower airways of OA-sensitised guinea pigs, it has been reported that oral administration of ONO-1078 at doses of more than 3 mg/kg, significantly reduced antigen-induced microvascular leakage in intrapulmonary airways (Obata et al., 1992). In studies of allergic rhinitis patients, leukotrienes have been shown to cause nasal congestion (Kojima et al., 1991; Naclerio et al., 1991) and increased blood flow (Naclerio et al., 1991) although nasally instilled LTD_4 failed to cause sneezing or rhinorrhea (Naclerio et al., 1991) . These results suggest that LTD_4 acts by stimulating specific receptors on end organs and not by reflex stimulation alone. However, it has been shown that LTD_4 receptor antagonist, ICI 204219 relieved the symptoms (including both sneezing and rhinorrhea) of allergic rhinitis (Donnelly et al., 1995). Further study will be necessary to determine whether or not Cys LTs are involved in the sneezing and rhinorrhea of allergic rhinitis. In summary, the present study revealed that a Cys LT antagonist (ONO-1078) inhibited both LTD_4 - and antigen-induced microvascular leakage in guinea pigs. However, a species difference must be taken into consideration in extrapolation to the therapeutic use of LT receptor antagonists in patients with allergic rhinitis.

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ANNOUNCEMENT