Involvement of endogenous tachykinins in LTD₄-induced airway responses


ABSTRACT: Leukotriene D₄ (LTD₄) has been reported to cause tachykinin release from airway sensory nerves. However, the functional significance of endogenously released tachykinins in LTD₄-mediated airway responses has not been fully clarified.

The aim of this study was to investigate whether LTD₄-induced airway responses are due, in part, to tachykinin release in guinea-pigs. Airway plasma exudation and bronchoconstriction were assessed by measuring extravasation of Evans blue dye and by mean pulmonary resistance (Rₕ) in the presence of atropine (1 mg·kg⁻¹ i.v.) and propranolol (1 mg·kg⁻¹ i.v.), respectively.

LTD₄ (5 µg·mL⁻¹ for 1 min) inhalation caused increase in plasma exudation and Rₕ. Capsaicin pretreatment of animals to deplete sensory neuropeptides significantly inhibited LTD₄-induced plasma exudation in the main bronchi, but not in the central (cIPA) and peripheral intrapulmonary airways (pIPA). Pretreatment with specific tachykinin neurokinin-1 (NK₁)-receptor antagonists, FK 888 (10 mg·kg⁻¹ i.v.) and CP 96345 (4 mg·kg⁻¹ i.v.), also significantly reduced LTD₄-induced plasma exudation in the main bronchi, and in the main bronchi and cIPA, respectively. However, these antagonists did not significantly affect the LTD₄-induced increase in Rₕ. In contrast, neurokinin-2 (NK₂)-receptor antagonist, SR 48968 (0.3 mg·kg⁻¹ i.v.), significantly inhibited the bronchoconstriction after LTD₄-inhalation.

These results suggest that leukotriene D₄-induced bronchoconstriction and plasma exudation in guinea-pigs are, in part, due to tachykinin release from airway sensory nerves.

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Methods

Animal preparations

Male Dunkin-Hartley guinea-pigs (n=70; Funabashi Farm, Funabashi, Japan) weighing 350–450 g were anaesthetized with an intraperitoneal injection of urethane (2 g·kg⁻¹). Animals were placed on a heated pad (Deltaphase Isothermal Pad, model 39DP, Braintree Science Inc., UK) to maintain the body temperature at about 37°C. Both external jugular veins were exposed for administration of drugs. All animals were pretreated 30 min before experimentation with atropine and propranolol (1 mg·kg⁻¹ i.v., for both) to block muscarinic and β-adrenergic receptor-mediated modification, respectively. The doses of atropine and propranolol were chosen according to previous studies [10, 11]. A tracheal cannula was inserted into the lumen of the cervical trachea, and this cannula was connected to a constant volume mechanical ventilator (Model SN-480-7, Shinano Seisakusho, Tokyo, Japan) at a frequency of 60 strokes·min⁻¹ and at a tidal volume (VT) of 1 mL·100 g⁻¹.

Pulmonary resistance

Airflow (V’) was determined by connecting a pneumotachograph (Model 00; Fleisch, Lausanne, Switzerland) to the tracheal cannula and measuring the pressure drop across the device with a differential pressure transducer (MP45, ±5 cmH₂O; Validyne Corp., Northridge, CA, USA). VT was determined by electrical integration of the airflow signal. Pleural pressure (Pₚp) was measured by means of an oesophageal balloon connected to the differential pressure transducer (MP45, ±100 cmH₂O). Transpulmonary pressure (Pₚt) was obtained by electrical subtraction of Pₚp from airway opening pressure (Pₚo). Pulmonary resistance (RL) was obtained by a subtraction method [12]. RL change was monitored after LTD₄ inhalation for 5 min.

Measurement of airway plasma exudation

Vascular permeability was quantified by the extravasation of Evans blue dye, which correlates well with the extravasation of radiolabelled albumin in guinea-pig airways [13]. Evans blue dye (30 mg·kg⁻¹, 30 mg in 1 mL saline) which was filtered using a 0.22 µm Millipore filter, was injected into the jugular vein 1 min before LTD₄ (5 µg·kg⁻¹ for 1 min) or saline (for 1 min) inhalation. The inhalation challenge was performed using an ultrasonic nebulizer (mean particle size = 5 µm; manufacturer's specification) (NE-U11B; Onron, Tateishi Co., Tokyo, Japan) interposed between the inspiratory part of the ventilator and the tracheal cannula. After the induction of leakage (5 min after the LTD₄ challenge), the thorax was opened and a catheter was inserted into the aorta through a left ventriculotomy. Ventricles were cross-clamped, and blood was expelled through an incision in the right atrium at 80 mmHg pressure with about 100 mL saline (pH 5.5) until the perfusate became clear. The lungs were removed, and parenchyma was scraped off. The main bronchi and the intrapulmonary airways were separated from each other. The intrapulmonary airways were divided into central (cIPA, the proximal 3 mm portion) and peripheral (pIPA, the remaining distal portion) components as described previously [10, 11]. All tissues were blotted dry and weighed. Evans blue dye was extracted in 2 mL of formamide at 37°C incubation for 16 h. Dye concentration was quantified from light absorbance at 620 nm (Spectrophotometer 220A; Hitachi Ltd., Tokyo, Japan) and expressed as ng dye per mg wet weight of tissue, as calculated from a standard curve of dye concentrations in the range of 0.5–10 µg·mL⁻¹.

Capsaicin pretreatment

Animals were anaesthetized with ketamine (50 mg·kg⁻¹ i.m.) and xylazine (0.1 mg·kg⁻¹ i.m.). Aminophylline (25 mg·kg⁻¹ i.p.) and terbutaline (0.1 mg·kg⁻¹ s.c.) were given 30 min before capsaicin administration to protect against bronchoconstriction. Capsaicin (50 mg·kg⁻¹ s.c.) or vehicle for capsaicin (ethanol/Tween 80, 1 mL·kg⁻¹ s.c.) was injected, and animals were studied 1 week after treatment.

Experimental protocols

The effects of intravenously administered neurokinin-1 (NK₁) antagonist, FK 888, or LTD₄ antagonist, ONO-1078, on LTD₄-induced Evans blue dye exudation were studied in seven groups: Group I: dimethylsulphoxide (DMSO, vehicle for both antagonists, 0.1 mL·kg⁻¹) and saline inhalation (n=5); Group II: FK 888 (10 mg·kg⁻¹) and saline inhalation (n=5); Group III: ONO-1078 (200 µg·kg⁻¹) and saline inhalation (n=5); Group IV: DMSO and LTD₄ inhalation (n=6); Group V: FK 888 (1 mg·kg⁻¹) and LTD₄ inhalation (n=6); Group VI: ONO-1078 (10 mg·kg⁻¹) and LTD₄ inhalation (n=7); Group VII: ONO-1078 (200 µg·kg⁻¹) and LTD₄ inhalation (n=5). DMSO, FK 888 or ONO-1078 was administered 1 min before Evans blue dye injection (30 mg·kg⁻¹ i.v.). LTD₄ or saline inhalation followed 1 min after the injection of Evans blue dye. In a separate set of experiments, the effects of another NK₁-receptor antagonist, CP 96345 (4 mg·kg⁻¹ i.v.), neurokinin-2 (NK₂)-receptor antagonist, SR 48968 (0.3 mg·kg⁻¹ i.v.), or vehicle of both antagonists, DMSO (0.1 mL·kg⁻¹ i.v.; n=5) on LTD₄-induced airway responses were studied. The doses of FK 888, CP 96345, and SR 48968 were chosen according to previous studies [14–16]. In a separate experiment, guinea-pigs chronically pretreated with capsaicin (n=8) or the vehicle of capsaicin (n=8) were challenged with LTD₄ inhalation to examine the effect of tachykinin depletion on the LTD₄-induced response.

Drugs and chemicals

FK 888 and ONO-1078 were kindly donated by Fujisawa Pharmaceutical Co. Ltd (Osaka, Japan) and Ono Pharmaceutical Co. Ltd (Osaka, Japan), respectively. CP 96345 was obtained from Yamanouchi Pharmaceutical Co. Ltd. (Ibaraki, Japan) and SR 48968 was kindly donated by X. Emonds-Alt (Sanofi Recherche, France). Evans blue dye was purchased from Aldrich Chemical Co. Inc.
(Milwaukee, WIS, USA). Substance P, urethane and xylazine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dimethylsulphoxide (DMSO), ethanol, formamide and Tween 80 were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Atropine sulphate was obtained from Tanabe Chemical Co. (Osaka, Japan). Propranolol hydrochloride was purchased from Imperial Chemical Industries plc (Macclesfield, UK). Five percent Glucose and saline (0.9% sodium chloride) were obtained from Otsuka Chemical Co. (Tokyo, Japan). Aminophylline was purchased from Eisai Co. Ltd (Tokyo, Japan). Ketamine was obtained from Sankyo Co. Ltd (Tokyo, Japan).

FK 888 and ONO-1078 were dissolved in 100% DMSO at the concentration of 100 and 2 mg mL\(^{-1}\), respectively, and these antagonists were injected by microsyringe (MS-N50; Terumo, Tokyo, Japan) at a volume of 0.1 mL kg\(^{-1}\). Capsaicin was diluted to a concentration of 50 mg mL\(^{-1}\) in 10% ethanol, 10% Tween 80 and 80% saline.

**Statistical analysis**

Data are expressed as mean±SEM. Comparisons of the concentration of extractable Evans blue dye among groups were made with Student’s unpaired t-test with Bonferroni correction. The degree of LTD\(_4\)-induced bronchoconstriction was analysed by two-way analysis of variance between groups, and Student’s unpaired t-test. Probability values of less than 0.05 were considered significant.

**Results**

**Effect of ONO-1078 on LTD\(_4\)-induced plasma exudation**

The tissue content (mean±SEM) of Evans blue dye in response to saline inhalation challenge (basal plasma exudation) in main bronchi, cIPA and pIPA was 13.8±3.3, 18.9±8.8 and 17.4±4.6 ng mg\(^{-1}\) tissue, respectively (fig. 1). LTD\(_4\) inhalation significantly increased Evans blue dye exudation in main bronchi to 127.1±16.2, in cIPA to 124.9±8.4, and in pIPA to 178.3±18.2 ng mg\(^{-1}\) tissue. ONO-1078 (200 µg kg\(^{-1}\) i.v.) itself had no significant effect on the basal plasma exudation in any component of the airways, but almost completely inhibited the LTD\(_4\)-induced plasma exudation in all airways (fig. 1).

**Effect of FK 888 on LTD\(_4\)-induced plasma exudation**

FK 888 (10 mg kg\(^{-1}\) i.v.) itself also had no significant effect on basal extravasation in any part of the airways (fig. 2). LTD\(_4\) inhalation-induced plasma exudation was significantly reduced by intravenous injection of FK 888 at the dose of 10 mg kg\(^{-1}\) in main bronchi but not in intrapulmonary airways (fig. 2).
Effect of CP 96345 and SR 48968 on LTD₄-induced plasma exudation

CP 96345 (4 mg·kg⁻¹ i.v.) significantly inhibited LTD₄-induced airway plasma exudation in main bronchi and cIPA, but not in pIPA (fig. 3). SR 48968 (0.3 mg·kg⁻¹ i.v.) did not have a significant effect on LTD₄-mediated plasma exudation in any airways (fig. 3).

Effect of the capsaicin pretreatment on LTD₄-induced plasma exudation

In the vehicle for capsaicin pretreated animals, LTD₄ inhalation-caused Evans blue dye exudation in main bronchi, cIPA and pIPA of 140.0±20.5, 133.5±12.1 and 197.3±23.0 ng·mg⁻¹ tissue, respectively. This exudation was significantly reduced by capsaicin pretreatment in main bronchi but not in intrapulmonary airways (fig. 4).

Effect of ONO-1078 and FK 888 on the LTD₄-induced changes in pulmonary resistance

LTD₄ inhalation challenge significantly increased Rₐ at 1–5 min after the challenge (fig. 5). ONO-1078 (200 µg·kg⁻¹ i.v.) significantly inhibited Rₐ changes evoked by LTD₄ challenge. On the other hand, FK 888 (10 mg·kg⁻¹ i.v.) did not significantly affect the LTD₄-induced Rₐ changes (fig. 5).
vascular leakage by LTD₄ has been demonstrated using onse is LT receptor specific. In guinea-pigs, airway micro-
vascular leakage, indicating that this resp-
tion causes microvascular leakage from central to peri-
hal airways. Thus, in central airways, the LTD₄-
mediated airway microvascular leakage is more predominant in central and mid-airways than in distal airways [34, 35]. Taken together, LTD₄ inhalation may cause airway sensory nerve stimulation and result in the release of endogenous SP mainly in the central airways. NK₁ receptors have been localized to postcapillary venules which are the leaky sites [27, 28]. NK₁ receptors have been demonstrated to be localized to postcapillary venules which are the leaky sites [28, 29]. Further, the NK₁-receptor antagonist, FK 888, has been reported to abolish sensory nerve stimulation [30] or exogenous SP [31]-mediated responses. In the present study, both pretreatments (capsaicin desensitization and NK₁-receptor antagonist pretreatment) significantly inhib-
ited LTD₄-mediated airway microvascular leakage in central airways, suggesting that, in this portion, the LTD₄-
mediated airway microvascular leakage is, in part, medi-
ated via tachykinin release from sensory nerves.

Administration of LTD₄ into the airways has been shown to enhance the recovery of SP- and NKA-like immunoreactivities from the lung, suggesting that LTD₄ causes the tachykinin release from airway sensory nerves [8]. However, conflicting results have been reported on the role played by tachykinins in the LTD₄-mediated responses in different tissues. In guinea-pig ileum, the LTD₄-mediated contractile response is partially inhibi-
ted by a neurotoxin, tetrodotoxin. This inhibitory effect is abolished by capsaicin desensitization or pretreatment with an SP antagonist, indicating that SP is released from neurones by LTD₄ in guinea-pig ileum [7]. In contrast, MANZINI and MEINI [32] have reported that capsaicin desensitization does not affect the bronchial contraction elicited by LTD₄ in guinea-pigs. The results of our pre-
sent investigation are compatible with the former study.

In the present study, both capsaicin desensitization and NK₁-receptor antagonist pretreatment inhibited the LTD₄-
induced airway microvascular leakage in central but not in peripheral airways, suggesting that tachykinin-mediated mechanisms are involved in LTD₄-induced inflammation in central rather than peripheral airways. SP-immunore-
active nerves are distributed in all airways [33], but sen-
sory nerve-stimulation-induced airway microvascular leakage is more predominant in central and mid-airways than in distal airways [34, 35]. Taken together, LTD₄ inhalation may cause airway sensory nerve stimulation and result in the release of endogenous SP mainly in the central airways. Thus, in central airways, the LTD₄-induced airway microvascular leakage is due to the activation of NK₁- and LTD₄-receptors on the endothelium of airway postcapillary venules. In contrast, in peripheral airways, LTD₄-mediated response may be due to LTD₄- but not NK₁-receptors. A similar effect of endoge-
nous tachykinins in central airways after bradykinin administration into the airways has also been reported [14].

In the present study, we observed significant inhibi-
tory effects of FK 888 only in the high dose range com-
pared with the former studies [30, 31]. Therefore, the nonspecific effect of this antagonist may influence our results. However, another NK₁-receptor antagonist, CP 96345, also showed a similar inhibitory effect on LTD₄-
induced responses. Thus, we believe that the effect of FK 888 observed in this study was a NK₁-receptor spe-
cific response.

In the present study, LTD₄ inhalation caused signifi-
cant bronchoconstriction. In contrast to the plasma leak-
age, NK₁-receptor antagonists, FK 888 or CP 96345, did
not affect the bronchoconstrictor response elicited by LTD₄ administration. However, NK₁-receptor antagonist, SR 48968, significantly inhibited the LTD₄-induced RL elevation. These results are in keeping with observations in humans in vivo, showing a slight increase in maximal bronchoconstriction to LTD₄ following pretreatment with an inhibitor (thiorphan) or tachykinin-degrading enzyme, neutral endopeptidase [36]. Because tachykinins have been reported to cause airway smooth muscle contraction via NK₁-receptors [6, 27], the LTD₄-induced RL elevation observed in the present study seems to be due to the airway smooth muscle contraction rather than airway plasma leakage and subsequent airway wall oedema.

In summary, we have shown that LTD₄ inhalation causes airway microvascular extravasation and bronchoconstriction. Both chronic capsaicin administration, which causes tachykinin depletion, and NK₁-receptor antagonist pretreatment partially but significantly reduced the plasma exudation in central airways. Furthermore, NK₁-receptor antagonist significantly inhibited the LTD₄-induced bronchoconstrictor response. These results suggest that the LTD₄-induced airway responses are, in part, mediated via tachykinin release from airway sensory nerves.

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