CysLT₂ receptor activation is involved in LTC₄-induced lung air-trapping in guinea pigs

Tomohiko Sekioka, Michiaki Kadoda, Yasuo Yonetomi, Akihiro Kamiya, Manabu Fujita, Takeshi Nabe, Kazuhito Kawabata

A b s t r a c t

CysLT₁ receptors are known to be involved in the pathogenesis of asthma. However, the functional roles of CysLT₂ receptors in this condition have not been determined. The purpose of this study is to develop an experimental model of CysLT₂ receptor-mediated LTC₄-induced lung air-trapping in guinea pigs and use this model to clarify the mechanism underlying response to such trapping. Because LTC₄ is rapidly converted to LTD₄ by γ-glutamyltranseptidase (γ-GTP) under physiological conditions, S-hexyl GSH was used as a γ-GTP inhibitor. In anesthetized artificially ventilated guinea pigs with no S-hexyl GSH treatment, i.v. LTC₄-induced bronchoconstriction was almost completely inhibited by montelukast, a CysLT₁ receptor antagonist, but not by BayCysLT₂RA, a CysLT₂ receptor antagonist. The inhibitory effect of montelukast was diminished by treatment with S-hexyl GSH. The air-trapping effect of BayCysLT₂RA was enhanced with increasing dose of S-hexyl GSH. In conclusion, CysLT₂ receptors mediate LTC₄-induced air-trapping.

1. Introduction

Cysteinyl leukotrienes (CysLTs: LTC₄, LTD₄ and LTE₄) are inflammatory lipid mediators that elicit various pathophysiological events, including bronchoconstriction, vascular hyperpermeability, mucus secretion, and inflammatory cells influx (Liu and Yokomizo, 2015) via activation of CysLT₁ receptors. Accordingly, a number of CysLT₁ receptor antagonists are currently used for treatment of asthma (Choby and Lee, 2015). CysLT₂ receptors are known to be expressed in the bronchial epithelium, smooth muscles, and on leukocytes (Heise et al., 2000; Corrigan et al., 2005; Mita et al., 2001). In addition, we have previously reported that both CysLT₁ and CysLT₂ receptors are expressed in airway tissues isolated from bronchial asthma subjects (Sekioka et al., 2015). Thus, activation of CysLT₂ receptors is expected to contribute to the pathogenesis of asthma.

Asthmatic airway obstruction is characterized by increased expiratory resistance, which elicits airways narrowing by bronchospasm, mucosal edema, mucus hypersecretion, and other inflammatory changes (DeGiorgi and White, 2008). Severe airflow obstruction in asthma patients is closely associated with insufficient expiration from the lungs, leading to air-trapping in the alveoli (DeGiorgi and White, 2008). This air-trapping may be mediated via CysLTs, considering the pathophysiologival functions of these cysteinyl leukotrienes. Indeed, montelukast, a CysLT₁ receptor antagonist, has been reported to improve regional air-trapping due to small airflow obstruction in asthma patients (Zeidler et al., 2006). However, it has been unclear whether asthma-associated air-trapping can be effectively treated with CysLT₁ receptor antagonists. As for CysLT₂ receptors, their role in asthma air-trapping has so far not been clarified.

Guinea pigs have been used as experimental animals for the development of LT modifiers, because their airway smooth muscles respond well to CysLTs. In guinea pigs, CysLT₂ receptors contribute to asthmatic response involving air-trapping.

Keywords:
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activated by LTC₄, whereas LTD₄ is known to potently activate CysLT₁ receptors (Ito et al., 2008). In humans, on the other hand, LTC₄ and LTD₄ are reported to have similar binding affinity for human CysLT₂ receptors (Nothacker et al., 2000). Because LTC₄ is rapidly metabolized to LTD₄ by γ-glutamyl transpeptidase (γ-GTP) (Orning and Hammarström, 1980; Synder et al., 1984), LTC₄-induced bronchoconstriction in guinea pigs is preferentially mediated via CysLT₁ receptors. Thus, in order to make an animal model with asthmatic response mediated via CysLT₂ receptors, a number of γ-GTP inhibitors have been used to minimize LTC₄ metabolism to LTD₄ (Bäck et al., 2001). We have recently reported that pre-treatment with S-hexyl GSH, a synthetic substrate of γ-GTP, in guinea pigs promotes LTC₄- or antigen-induced bronchoconstriction and/or airway vascular hyperpermeability via both CysLT₁ and CysLT₂ receptors (Yonetomi et al., 2015a, 2015b).

The purposes of this study is to develop an experimental model of CysLT₂ receptor-mediated LTC₄-induced lung air-trapping in guinea pigs using S-hexyl GSH, and to clarify the mechanism underlying response to such trapping using montelukast, a CysLT₁ receptor antagonist, BayCysLT₂RA, a CysLT₂ receptor antagonist, and salmeterol, a bronchodilatory adrenergic β₂ agonist.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Japan SLC, Shizuoka, Japan) aged 6–7 weeks were used in this study. They were housed in air-conditioned room maintained at 24 ± 2 °C and 55 ± 5% relative humidity with alternating 12 h light/dark cycle, and were provided with food (LRC4, Oriental Yeast Co., Ltd., Japan) and tap water ad libitum. All animal experiments were approved by the Animal Ethical Committee of Ono Pharmaceutical Co., Ltd., and were performed in accordance with the institutional animal care guidelines.

2.2. Drugs and materials

Montelukast, a CysLT₁ receptor antagonist, montelukast was purchased from Sequoia Research Products, Ltd. (Pangbourne, UK). BayCysLT₂RA; 1-[5-carboxy-2-{3-[4-(3-cyclohexylpropoxy)phenyl]propoxy}benzoyl]-4-piperidincarboxylic acid (Härter et al., 2004), was synthesized in our laboratories and used as CysLT₂ receptor antagonist. Other materials used in this study were purchased commercially: LTC₄ and LTD₄ (Cayman Chem., MI, USA), S-hexylglutathione (S-hexyl GSH), salmeterol xinafoate, fluorescein isothiocyanate-conjugated bovine serum albumin (FITC-BSA) and fluorescein isothiocyanate-conjugated human serum albumin (FITC-HSA) (Sigma-Aldrich, St. Louis, MO, USA).

Montelukast (0.1 or 0.3 mg/kg) suspended in 0.5% methylcellulose solution (Wako Pure Chem., Osaka, Japan) was orally administered to guinea pigs 24 h before LTC₄ or LTD₄ administration. BayCysLT₂RA (1 mg/kg) was dissolved in physiological saline containing 1% Wellsolve (Celeste Co., Tokyo, Japan). Salmeterol (30 µg/kg) was dissolved in physiological saline containing 2% N,N-dimethylformamide. BayCysLT₂RA and salmeterol were intravenously (i.v.) administered to guinea pigs 1 min before LTC₄ or LTD₄ administration. The dose of montelukast was selected based on the results of our previous study showing that montelukast, given orally at 0.1 and 0.3 mg/kg, completely inhibit LTD₄ (1 µg/kg, i.v.)-induced airway vascular hyperpermeability and LTD₄ (0.5 µg/kg, i.v.)-induced bronchoconstriction in guinea pigs, respectively (Yonetomi et al., 2015b). As for the selected dose of BayCysLT₂RA, we have reported that intravenous administration of this CysLT₂ receptor antagonist (described as Compound A) at 1 mg/kg (i.v.) significantly attenuates LTC₄ (15 µg/kg, i.v.)-induced bronchoconstriction in S-hexyl GSH-treated guinea pigs (Yonetomi et al., 2015a). In addition, we have found in an acute toxicity study that BayCysLT₂RA (3 mg/kg, i.v.) induces hemolysis in 1 out of 8 guinea pigs (unpublished data). Finally, the dose of salmeterol was selected based on the fact that salmeterol at 30 µg/kg completely suppresses LTD₄-induced bronchoconstriction (data not shown).

2.3. i.v. LTC₄-induced bronchoconstriction in anesthetized artificially ventilated guinea pigs

I.v. LTC₄-induced bronchoconstriction in anesthetized artificially ventilated guinea pigs was assessed according to the method of Konzett and Rössler (1940). Briefly, guinea pigs were anesthetized with pentobarbital sodium (75 mg/kg, i.p.). One end of a polyethylene cannula was inserted into the trachea, and the other end was connected to a volume-limited ventilator (Model SN-480-7, Shinnano Manufacturing Co., Ltd., Tokyo, Japan). Artificial ventilation was provided at a rate of 70 strokes/min with 4 ml/stroke. Another catheter was inserted into the jugular vein, securing the route for administration. Ventilation pressure was measured via a pneumotachometer (M.I.P.S Co., Ltd., Osaka, Japan) connected to the lateral port of the tracheal cannula, using a Win-PULMOS-III system (Version 3.6, M.I.P.S Co., Ltd., Osaka, Japan). After stabilization of basal ventilation pressure, LTC₄ (10 µg/kg) was i.v. administered via the catheter secured in the jugular vein. S-hexyl GSH was i.v. administered 10 s before LTC₄ administration. Following measurement of ventilation pressure for 30 min after LTC₄ dosing, the trachea was completely blocked to obtain maximum ventilation pressure. After measurement of maximum ventilation pressure, mechanical ventilation was stopped, and the animal was euthanized. The area under the bronchoconstriction curve (AUC) from 0 to 30 min after LTC₄ challenge was calculated by the trapezoidal method, and mean percentage bronchoconstriction (%) was calculated by dividing AUC by measurement time.

2.4. Inhaled LTC₄ and LTD₄-induced bronchoconstriction in conscious guinea pigs

Inhaled LTC₄ or LTD₄-induced bronchoconstriction in conscious guinea pigs was assessed according to the method of Pennock et al. (1979). Specific airway resistance (sRaw), as bronchoconstriction indicator, was measured 5–60 min after LTC₄ or LTD₄ inhalation using a two-chambered, double-flow plethysmograph system (Pennock et al., 1979). The animal was placed with its neck extending through the partition of a two chambered box, and sRaw was measured with a Data analyzer Win-PULMOS-III system (Version 3.6, M.I.P.S Co., Ltd., Osaka, Japan). Animals that showed severe dyspnea or cyanosis after LTC₄ or LTD₄ inhalation were humanely euthanized by pentobarbital sodium overdose.

2.5. Measurement of whole lung volume

After measurement of sRaw, the animals were first anesthetized with pentobarbital sodium (75 mg/kg, i.p.) and then exsanguinated by cutting the abdominal aorta and vena cava. Thoracotomy was next performed, and the whole lung, together with the trachea and bronchial cannula, were isolated. The lung lobes were separated, and whole lung volume was measured by a plethysmometer (TK-101 CMP, Unicom, Chiba, Japan).

2.6. Lung histological examination

Lung tissues were fixed in 10% formaldehyde, embedded in paraffin wax, and cut into 4-μm thick sections. The sections were stained with hematoxylin and eosin (HE) for histological examination.

2.7. Measurement of airway vascular permeability

Immediately after the intravenous administration of FITC-BSA...
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