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Montelukast inhibits inflammatory responses in small airways of the Guinea-pig

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Abstract

Increased resistance in the small airways is a major contributor of airway obstruction in asthma. The role of leukotrienes (LT) in determining inflammation and obstruction of small size bronchi is not completely understood. Here, we have examined the effect of the cysteinyl-leukotriene (*Cys*LT_1) receptor antagonist, montelukast, against the bronchoconstriction and inflammatory responses induced by exogenous leukotriene and by allergen challenge in small size ($\leqslant 1$ mm) Guinea-pig bronchi. Montelukast potently (pA_2 8.3) inhibited the contraction induced by LTD_4 in small bronchi taken from naïve Guinea-pigs. Furthermore, montelukast reduced the contraction produced by in vitro ovalbumin (OVA) challenge in small size bronchi from sensitized Guinea-pigs. Montelukast ($10\,\mu g\,kg^{-1}$) also blocked plasma protein extravasation and accumulation of inflammatory cells (eosinophils) induced by OVA challenge in small intraparenchymal bronchi of OVA sensitized animals. These findings provide additional evidence that *Cys*LT_1 receptor antagonism reduces allergic reactions that cause contractile and inflammatory responses in Guinea-pig small airways during OVA challenge. If the anti-bronchospastic and anti-inflammatory actions of the *Cys*LT_1 receptor antagonists observed in the small airways of Guinea-pigs occur also in man these effects may contribute to the beneficial effects of montelukast in asthmatic patients.

Keywords: Inflammation; Leukotriene; Montelukast; Ovalbumin challenge; Resistance; Small airways

1. Introduction

Asthma is a chronic inflammatory disorder with recurrent episodes of coughing, wheezing, chest tightness and breathlessness, produced by bronchoconstriction, airway oedema, mucus secretion and airway remodelling [1]. Some mediators, which include histamine, prostaglandin D_2 and cysteinyl-leukotrienes (*Cys*LTs), are primarily involved in the early phase of the allergic bronchoconstrictive response [2–4]. In particular, the effects of *Cys*LTs are thought to contribute to bronchoconstriction, increased mucus secretion and decreased mucociliary clearance [5]. Furthermore, an increase in *Cys*LTs has been detected in the urine [6], bronchoalveolar lavage (BAL) fluid [7] and sputum [8] of patients with asthma. There is evidence for the presence of, at least, two *Cys*LT receptors (*Cys*LT_1 and *Cys*LT_2) in the lung [9,10]. A number of inhibitors of leukotriene biosynthesis or receptor antagonists for leukotriene receptors have been investigated in preclinical and clinical studies [11], including montelukast, a potent and specific antagonist of *Cys*LT_1 receptor [12–14].

Airflow limitation in asthma results from a number of pathophysiological changes, including smooth muscle contraction, airway wall oedema, mucus hypersecretion, bronchial wall remodelling and other components [15].

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A prominent inflammatory response in the distal lung is a well-known feature of human asthma [16], which may have important functional consequences, as demonstrated by pioneering studies on the role of peripheral tissue resistance in asthmatics [17,18]. Previous studies have proposed that the increased resistance to airflow seen during an asthma attack or following bronchial challenge is caused by contraction of both proximal and distal airways [19,20]. Recent evidence suggests that leukotriene D_4 (LTD_4) causes remarkable contraction of human small bronchioles by activation of *Cys*LT_1 receptors [21,22]. In Guinea-pigs, *Cys*LT_1 receptors mediate LTD_4 induced plasma protein extravasation and bronchoconstriction both in large and small airways [23,24]. Despite these results, the precise contribution of *Cys*LTs and their receptors in decreasing the lumen of small size bronchi in animal models of allergic asthma has not been fully investigated. Thus, the purpose of the present study was to explore the contribution of *Cys*LTs to the increase bronchial tone and oedema in a Guinea-pig model of allergic airway inflammation. To this aim, we studied the effect of a *Cys*LT_1 receptor antagonist, montelukast, on: microvascular leakage; eosinophil accumulation; and bronchoconstriction produced by ovalbumin (OVA) administration in small size (<1 mm) bronchi in vivo and in vitro.

2. Methods

2.1. Animals

Male Dunkin-Hartley Guinea-pigs (250–350 g, Pampaloni, Pisa, Italy) were acclimatized in cages, (24 ± 0.5 °C) for 1 week after delivery, and were allowed free access to water and standard rodent diet (Morini, Italy). The study conformed to the Declaration of Helsinki, complied with the Italian guidelines and was approved by the local ethical committee for animal studies.

2.2. Ovalbumin sensitization and challenge protocol

Male Dunkin-Hartley Guinea-pigs were actively sensitized by a unique intra-peritoneal injection of 0.5 ml of 0.9% NaCl solution containing 10 μg of OVA (Grade V, Sigma), dispersed with 1 mg of $Al(OH)_3$. After fourteen days from the OVA administration, antigen challenge was performed in vivo by the i.v. administration of 100 μg kg^{-1} of OVA or in vitro by challenging with 1 μg ml^{-1} of OVA. Controls received, in both cases, the vehicle of OVA (0.9% NaCl).

2.3. Isolated Guinea-pig bronchi

2.3.1. Leukotriene D_4 (LTD_4)- and carbachol (CCH)-induced contraction of Guinea-pig isolated small bronchi

Guinea-pigs were terminally anesthetised with diethyl ether and the airways removed. Rings from small bronchi (≤1 mm diameter) were suspended under a resting tension of 1 g. Changes in contractile and relaxant tone were measured with an isometric force transducer (model 7003, Ugo Basile, Italy) and recorded on a polygraph (Unirecord 7050, Ugo Basile, Italy). Tissues were bathed and aerated (95% O_2 and 5% CO_2) with Krebs solution which was maintained at 37 °C. Tissues were allowed to equilibrate for 60 min prior to the beginning, and between each set of experiments (washed every 10 min). In all experiments tissues were first contracted with CCh (1 μM) to check tissue viability. Cumulative concentration-response curves were performed with LTD_4 (0.01 nM–1 μM) and CCh (1 nM–30 μM) and the effect of montelukast (0.01–1 μM) on this contractile response was studied.

2.3.2. Antigen-induced contraction of Guinea-pig isolated small bronchi

Small size bronchi were taken from OVA-sensitized Guinea-pigs or vehicle control animals (0.9% NaCl). Rings were placed in 5 ml organ baths and challenged with OVA (1 μg ml^{-1}) in the presence of montelukast (0.1–1 μM) or its vehicle.

2.3.3. Plasma extravasation

Fourteen days following the OVA administration Guinea-pigs were anaesthetized (sodium pentobarbital, 45 mg kg^{-1} i.v.) and artificially ventilated (60 breaths min^{-1} with a tidal volume of 5 ml). Evans Blue (30 mg kg^{-1} i.v. over 5 s) was injected into the jugular vein one minute prior to the intravenous injection (jugular vein) of OVA (100 μg kg^{-1}, in 0.9% NaCl, 1 ml kg^{-1}) or vehicle (0.9% NaCl, 1 ml kg^{-1}). In a sub-set of animals, 15 min prior to the OVA challenge, an intravenous injection of montelukast (10 μg kg^{-1}), or its vehicle (saline), was performed. After fifteen additional minutes the chest wall was open, animals were sacrificed and the circulating Evans blue dye removed by transcardial perfusion for 3 min with phosphate buffer at a pressure of 120 mm Hg. Small bronchi were removed, weighed and incubated in 1 ml of formamide for 24 h in the dark, at room temperature. The amount of extravasated Evans Blue was measured spectrophotometrically at 620 nm.

2.4. Histology

Lung specimens obtained from 7 Guinea-pigs pre-treated with montelukast (10 μg kg^{-1}) 30 min before OVA challenge and 6 Guinea-pigs challenged with OVA without pre-treatment (untreated controls) were examined. Lungs were removed 24 h after the OVA challenge, fixed in 4% formaldehyde in phosphate-buffered saline (PBS) at pH 7.2 and, after dehydration through an alcohol series, were embedded in paraffin wax. Sections (4–5 μm thick) were cut from each block and stained with haematoxylin–eosin to analyze the total and eosinophilic inflammatory infiltrate. For each animal, at least 5 small size airways (diameter-<1 mm) were identified. These airways had to be intact, transversally cut and should not contain cartilage or