BI-L-239, a 5-lipoxygenase inhibitor, blocks inhaled antigen-induced airway hyperresponsiveness in conscious guinea pigs

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Abstract
Male Hartley guinea pigs were actively sensitized to ovalbumin (OA). Respiratory system resistance (Rrs) was measured by forced oscillations superimposed on tidal breathing. Airway responsiveness (inhaled methacholine PC_{100}) was determined three days prior and three days after (day 10) three alternate day inhalations of OA. Airway cell composition was assessed on day 10 by lung lavage. Three groups (n = 5–6) were studied: A) vehicle challenged, B) OA challenged/placebo treated, C) OA challenged/BI-L-239 (2,6-dimethyl-4-[2-(4-fluorophenyl)ethenyl]phenol) treated (10 x 0.75 mg/actuation, 10 minutes prior to each OA challenge). Animals were treated with pyrilamine and indomethacin (10 mg/kg i.p.) 30 minutes prior to each OA challenge. OA induced acute increases in Rrs of 143 ± 29%, 238 ± 73% and 102 ± 43% in placebo and 86 ± 34%, 45 ± 35% (p, 0.05 vs. placebo) and 102 ± 31% in BI-L-239 treated. OA induced a significant (p < 0.05) increase in airway leukocytes in placebo (487 ± 36 to 1615 ± 421 x 10^3/ml) but not BI-L-239 treated (to 881 ± 155 x 10^3/ml) and decrease in methacholine PC_{100} in placebo (1.487 ± 0.49 to 0.39 ± 0.18 mg/ml) but not BI-L-239 treated (0.99 ± 34 to 1.04 ± 0.39 mg/ml). We conclude that BI-L-239 attenuates the airway constriction, inflammation and hyperresponsiveness induced by repeated antigen inhalations in conscious guinea pigs.

Introduction
Non specific airway hyperresponsiveness is a common feature found in patients with bronchial asthma [1]. In man [2], as well as in animals [3, 4], multiple antigen challenges have been shown to elucidate airway hyperresponsiveness. Recent interest has focused on the generation and accumulation of inflammatory products and cells in the airways and their relevance to the pathogenesis of asthma.

5-Lipoxygenase breakdown of arachidonic acid initiates the formation of leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) which induce bronchoconstriction, mucous secretion and increased vascular permeability [6]; and leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (5-HETE) which are potent chemotactants and activators of neutrophils and eosinophils [7]. The accumulation and activation of inflammatory cells in the airways can lead to epithelial cell damage and further the exposure of nerve endings in the bronchial lumen which, subsequently, leads to an increase in airway responsiveness [9].

The purpose of this study was to examine the effects of a 5-lipoxygenase inhibitor, BI-L-239 (2,6-dimethyl-4-[2-(4-fluorophenyl) ethenyl]phenol) [8], in a conscious guinea pig model of inhaled antigen (Ag)-induced airway hyperresponsiveness.
Methods and materials

Animals and Sensitization

Seventeen male Hartley guinea pigs (Charles River, Stone Ridge, N.Y.), weighing 270–370 grams at the time of Ag challenge, were actively sensitized to ovalbumin (OA) (Sigma, St. Louis, Mo.). Twenty-one days prior to the first Ag challenge each animal was injected i.p. with 1 mg OA plus 1 mg AL(OH)₃ (Amphojel, Wyeth Laboratories, Philadelphia, Pa.) in 1.0 ml 0.9% saline and with 5 x 10⁹ heat killed Bordetella pertussis (Institut Armand Frappier, Canada). The animals were boosted seven days later with one-tenth of the OA-AL(OH)₃ solution.

Pulmonary function monitoring

To evaluate pulmonary function repeatedly during the induction of acute and/or chronic airway/lung inflammation in a small animal, the following non-invasive measurement technique was developed for conscious guinea pigs. Each guinea pig was placed in a head out plethysmograph (Fig. 1). Using a sine wave generator, power amplifier and loudspeaker, a 10 Hz pressure signal was continuously induced in the plethysmograph. A nose cone connected to a three-way stopcock was then placed over the guinea pigs nose and mouth. With the stopcock open to a pneumotachograph, we measured the airflow induced and superimposed on tidal breathing by the 10 Hz pressure oscillation at the animal’s chest. This airflow and pressure were then used to calculate respiratory system impedance, resistance and reactance.

Experimental protocol

A 10 day protocol to induce inflammation was chosen based on previous studies done in primates [4]. Airway hyperresponsiveness [concentration of nebulized and inhaled methacholine to induce a 100% increase in respiratory resistance (Rrs), PC₁₀₀, to methacholine (Sigma, St. Louis, Mo) was determined three days prior to (day 0) and three days after (day 10), three alternate day (day 3, 5 and 7) inhalations of Ag. Following day 10 PC₁₀₀, the animals were terminated with sodium pentobarbital (Sigma) overdose, the trachea cannulated and a three aliquot whole lung lavage was performed to assess airway cell composition. Total cell counts with differentials were performed on each sample.

Three groups were studied: 1) phosphate buffered saline (PBS, antigen vehicle) challenged – untreated, 2) Ag challenged – placebo treated, and 3) Ag

Figure 1
Diagram illustrating aerosol administration and the measurement of Rrs by superimposed forced oscillations in conscious guinea pigs. Aerosols generated by the nebulizer are pulled through the aerosol mixing chamber and into the nose cone apparatus by a constant bias flow of air (3.0 l/min).