Effects of leukotriene B₄ receptor antagonist, LY293111Na, on antigen-induced bronchial hyperresponsiveness and leukocyte infiltration in sensitized guinea pigs

F. Asanuma¹, K. Kuwabara¹, A. Arimura¹, Y. Furue¹, J. H. Fleisch² and Y. Hori¹

¹ Discovery Research Laboratories, Shionogi & Co., Ltd., 3-1-1 Futaba-cho, Toyonaka, Osaka 561-0825, Japan, Fax: 81 6 6332 6385, e-mail: yozo.hori@shionogi.co.jp
² Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, USA

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Abstract. Objective and Design: LY293111Na, 2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy]-phenoxy]-benzoic acid sodium salt, is a novel leukotriene B₄ (LTB₄) receptor antagonist. Its effects on guinea pig models of asthma were compared with those of dexamethasone.

Methods: Effects of LY293111Na were tested in antigen (ovalbumin, OA)-induced bronchial hyperresponsiveness (BHR) and leukocyte accumulation in actively sensitized guinea pigs. Its effects on antigen-induced acute bronchoconstriction in passively sensitized guinea pigs were also studied.

Results: LY293111Na (10 to 30 mg/kg p.o., 1 h before and 6 h after OA challenge) inhibited BHR to acetylcholine. LY293111Na (3 mg/kg p.o.) significantly inhibited accumulation of neutrophils in bronchoalveolar lavage (BAL) fluid 24 h after antigen challenge but it did not inhibit accumulation of eosinophils and macrophages at any doses used. In contrast, dexamethasone (30 mg/kg p.o., 4 h before OA challenge) not only inhibited BHR but also reduced the infiltration of all three types of leukocytes. A significant increase of LTB₄ levels in BAL fluid was noted at 3 and 15 min after the antigen challenge. LY293111Na did not inhibit antigen-induced acute bronchoconstriction in passively sensitized guinea pigs.

Conclusion: These results indicate that LTB₄ may participate in antigen-induced BHR but not in eosinophil infiltration and acute bronchoconstriction in guinea pigs.

Key words: Leukotriene B₄ – LY293111Na – Dexamethasone – Bronchial hyperresponsiveness – Leukocyte infiltration

Introduction

Asthma is characterized by pulmonary inflammation with severe airway eosinophilia, accompanying enhanced responsiveness of the airways to various spasmogens [1]. Among the inflammatory mediators involved in asthma, leukotrienes (LTs) are thought to be important in relation to causing bronchoconstriction, leukocyte infiltration, edema, mucus secretion and bronchial hyperresponsiveness (BHR) [2]. They are formed by the action of 5-lipoxygenase (5-LOX) on arachidonic acid released from the cell membrane or perinuclear envelope [3]. In contrast to the apparent involvement in asthma of cysteinyl LTs as revealed by clinical efficacy of LTD₄ receptor antagonists, the participation of LTB₄, another 5-LOX product of arachidonic acid, is still unknown [3, 4]. LTB₄ has been found in the bronchoalveolar lavage (BAL) fluids of asthmatic patients [5]. This C-20 fatty acid is a potent chemoattractant of both neutrophils and eosinophils and may also produce BHR to spasmogens such as histamine or acetylcholine [6, 7]. The reduction of LTB₄ activity by its antagonists might lead to the control of airway inflammation and BHR. Based on these concepts, several LTB₄ antagonists have already been tested in animal models of antigen-induced airway inflammation and/or BHR, however, the results have been inconsistent. In one study, LTB₄ receptor antagonists inhibited airway eosinophilia in guinea pigs [8] but in others, they inhibited BHR but did not leukocyte infiltration [9, 10]. In another study, both BHR and neutrophil infiltration were prevented by an LTB₄ receptor antagonist in a primate model of asthma [11].

LY293111Na, 2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy]-phenoxy]-benzoic acid sodium salt, is a potent and specific LTB₄ receptor antagonist [12–14]. In vivo, this compound has been shown to inhibit airway inflammation induced by LTB₄ in primates and guinea pigs [15, 16], however, it has not yet been tested against clinically relevant antigen-induced models of asthma. In the current study, we examined in vivo efficacy of this agent on guinea pig models of asthma with particular interest in antigen-induced leukocyte infiltration into the lungs and BHR. Dexamethasone was employed as a reference anti-asthma agent with strong anti-inflammatory action that
represents steroid therapy for clinical asthma [17]. Furthermore, we measured the changes of LTB₄ levels in BAL fluids to confirm the possible involvement of LTB₄ in leukocyte infiltration and/or BHR in this model.

Materials and methods

Drugs and chemicals
LY293111Na, 2-(2-propyl-3-[3-(2-ethyl-4-(4-fluorophenyl)-5-hydroxy- phenoxy][propoxy]-phenoxy]-benzoic acid sodium salt, was synthesized at Lilly Research Laboratories (Indianapolis, IN). Acetyicholine chloride (ACh), dexamethasone, diphenhydramine chloride, gallamine triethiodide, ovalbumin (OA, grade III) and urethane were all purchased from Sigma Chemical Co. (St. Louis, MO). Pentobarbital sodium (Nembutal™) was from Abbott Laboratories (Chicago, IL). LY293111Na was dissolved in distilled water. Dexamethasone was suspended in 0.5% gum arabic solution. Diphenhydramine chloride, gallamine triethiodide, OA and urethane were dissolved in sterile 0.9% saline.

Animals
Male Hartley guinea pigs, weighing 350 to 600 g (Charles River Japan, Kanagawa, Japan), were used. All animal experiments conducted in the present study were approved by the Shionogi Animal Use and Care Committee.

Active sensitization procedure and antigen challenge
Experiments were performed as previously described [18]. Briefly, the animals were actively sensitized to OA for 10 min in an exposure chamber on two occasions, with a 7-day interval, by inhalation of aerosolized solution of 1% OA using an ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan). Seven days after the second exposure, each animal was placed in the exposure chamber and subjected to challenge by inhalation of aerosolized 1% OA solution for 5 min. To protect them from anaphylactic death, all animals were treated with diphenhydramine (10 mg/kg i.p.), a histamine H₁ receptor antagonist, at 10 min before the antigen challenge.

Measurement of antigen-induced BHR
At 24 h after the third antigen challenge, the animals were anesthetized with urethane (2.0 g/kg i.p.) and dose-response curves were constructed for ACh (3.1–100 μg/kg i.v.) as described previously [18]. BHR was assessed by determining the dose of ACh (μg/kg) needed to reach 200% insufflation pressure of the respective base line value (PD₂₀₀) by linear interpolation.

BAL and cytological examination
After obtaining dose-response curves to ACh, the animals used were sacrificed with an overdose of i.p. urethane. Ten ml of 0.9% sterile saline was slowly injected into the trachea, withdrawn, and re-injected twice. The total number of cells was determined with a hemocytometer with Turk’s stain, and the fluid was centrifuged at 200 g for 10 min. The cell pellet was resuspended in 200 μl of fetal bovine serum, and differential cell counts were undertaken with cytocentrifuged preparations (Cytospin 3; Shandon Southern Instruments, Pittsburgh, PA) stained with May–Grünwald-Giemsa. A minimum of 500 were counted and classified as neutrophils, macrophages and eosinophils based on normal morphological criteria.

Measurement of LTB₄
In another set of experiments, the time course changes of LTB₄ in BAL fluid were examined after the third OA challenge. BAL was performed on five groups of six actively sensitized animals and six unsensitized animals at 0 (before), 3, 15, 30 min, 1 h or 24 h after antigen challenge. BAL fluid, harvested by washing the airway three times with 10 ml of 0.9% sterile saline containing 10 U/ml heparin, 30 μg/ml of phenindone and 10 μg/ml of indomethacin to inhibit coagulation, 5-LOX and cyclooxygenase, respectively, during preparation. The sample was centrifuged at 100 g for 10 min at 4°C and the supernatant was stored at –80°C until assay. LTB₄ was extracted using a Sep-Pak C18 cartridge (Waters Associates, Milford, USA) as previously described [18] and the concentrations of those in the eluant of BAL fluid were measured with a radioimmunnoassay kit (Amersham, Little Chalfont, UK).

Passive sensitization procedure and antigen-induced acute bronchoconstriction
In a separate experiment, guinea pig anti-OA antiserum was prepared as described previously [19]. The anti-OA IgG antibody titer of antisera was 1:1,600 as determined by 4-h homologous passive cutaneous anaphylaxis [20]. Bronchoconstriction was measured as in the BHR experiments described above except that animals were anesthetized with pentobarbital sodium (30 mg/kg i.p.) instead of urethane. To suppress spontaneous breathing, the animals were treated with gallamine triethiodide (2 mg/kg i.v.) just after the surgical operation. OA-induced bronchoconstriction was elicited by intravenous administration of OA (40 or 70 μg/kg) in guinea pigs which had been passively sensitized with intraperitoneal injection of 0.2 ml of anti-OA serum 48 h prior to the antigen challenge. Forty μg/kg of OA was used in animals treated i.v. with mixed antagonists (diphenhydramine 5 mg/kg, indomethacin 10 mg/kg and propranolol 1 mg/kg; 5 min prior to OA challenge) to inhibit the bronchoconstrictor effect of endogenously released histamine and to enhance that of the endogenous 5-LOX products. In contrast 70 μg/kg OA was used in animals not treated with these mixed antagonists to obtain the similar magnitude of bronchoconstriction as in the animals with the mixed antagonists.

Statistical analysis
All values were represented as mean ± SEM. The statistical significance of the data was evaluated by unpaired t-test when only two value sets were compared, and by Dunnett’s test when the data involved three or more groups. Differences were considered significant if p < 0.05.

Results
Effects of LY293111Na and dexamethasone on antigen-induced BHR
Antigen challenge by an aerosol of 1% OA for 5 min to sensitized animals resulted in BHR to ACh 24 h later when compared to saline exposure of sensitized animals, as shown by a decrease of log [ACh PD₂₀₀] in OA-challenged animals (ACh PD₂₀₀ values = 12.7 ± 0.8 μg/kg, n = 10, p < 0.01) versus saline-challenged ones (27.6 ± 4.7 μg/kg, n = 8) (Fig. 1). LY293111Na, administered 1 h before and 6 h after the OA challenge, inhibited BHR at a dose of 10 mg/kg p.o. as assessed 24 h after the antigen challenge. A similar reduction in BHR was also noted after administration of 30 mg/kg LY293111Na. ACh PD₂₀₀ for 3, 10 and 30 mg/kg p.o. were 15.6 ± 1.6 (n = 8), 20.2 ± 2.5 (n = 10, p < 0.05) and 20.5 ± 17.4 (n = 8) (Fig. 2).