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Kinin B1 receptor antagonist BI113823 reduces allergen-induced airway inflammation and mucus secretion in mice

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ABSTRACT

Kinin B1 receptors are implicated in asthmatic airway inflammation. Here we tested this hypothesis by examining the anti-inflammatory effects of BI113823, a novel non-peptide orally active kinin B1 receptor antagonist in mice sensitized to ovalbumin (OVA). Male Balb-c mice were randomly assigned to four study groups: (1) control, (2) OVA + vehicle, (3) OVA + BI113823, (4) OVA + dexamethasone. Mice were sensitized intraperitoneally with 75 μg ovalbumin on days 1 and 8. On days 15–17, mice were challenged intranasally with 50 μg of ovalbumin. Mice received vehicle, BI113823, or dexamethasone (positive control) on days 16–18. On day 19, bronchoalveolar lavage (BAL) and lung tissue were collected for biochemical and immuno-histological analysis. Compared to controls treatment with BI113823 significantly reduced the numbers of BAL eosinophils, macrophages, neutrophils and lymphocytes by 58.3%, 61.1%, 66.4% and 56.0%, respectively. Mice treated with dexamethasone showed similar reductions in BAL cells. Treatment with BI113823 and dexamethasone also significantly reduced total protein content, IgE, TNF-α and IL-1β in lavage fluid, reduced myeloperoxidase activity, mucus secretion in lung tissues, and reduced the expression of B1 receptors, matrix metalloproteinase (MMP)-2 and cyclooxygenase (COX)-2 compared to vehicle-treated mice. Only BI113823 reduced MMP-9 and inducible nitric oxide synthase (iNOS). BI113823 effectively reduced OVA-induced inflammatory cell, mediator and signaling pathways equal to or greater than that seen with steroids in a mouse asthma model. BI113823 might be useful in modulating inflammation in asthma.

1. Introduction

Chronic airway inflammation is a characteristic feature of asthma [1] and kinins are important proinflammatory mediators that have been implicated in the pathogenesis of this airway inflammatory response [2,3]. Inflammatory cells are sources of tissue kallikrein [4] which increases kinin production. These increased kinin levels in the airways have been associated with airway inflammation, bronchoconstriction, increased vascular permeability, leukocyte activation and chemotaxis, synthesis of cytokines and chemokines, and mucus hypersecretion [3–9]. These actions of kinins occur through stimulation of two pharmacologically distinct receptor subtypes B1 and B2 [2]. Kinin B2 receptors are constitutively expressed, and are activated by bradykinin and kallidin; whereas kinin B1 receptors are induced by stimulation of lipopolysaccharides (LPS), IL-1β and TNF-α, by tissue injury and exposure to cigarette-smoke, and are activated by des-Arg9-bradykinin and des-Arg10-kallidin [2,3,7–10]. While both B1 and B2 receptors are implicated in pathogenesis of the airway inflammatory response in asthma [2,3,11], there is one important difference between the two subtypes. The B2 receptor is internalized rapidly and desensitizes, while the kinin B1 receptor is not internalized as indicated by the persistence of the B1-receptor ligand complex responses. Thus, kinin B1 receptors may be more influential than kinin B2 receptors in maintaining chronic inflammatory processes [8,12,13]. This is important because kinin B1 receptors are constitutively expressed in neutrophils, eosinophils, macrophages, dendritic cells, as well as in the epithelial cells and

Abbreviations: OVA, ovalbumin; BAL, bronchoalveolar lavage; COX, cyclooxygenase; MMP, matrix metalloproteinase; iNOS, inducible nitric oxide synthase; PAS, periodic acid of schiff; LPS, lipopolysaccharides; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin 1-beta; MPO, myeloperoxidase.

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fibroblasts of the human airways [8,14–18]. Collectively, these observations suggest that antagonizing kinin B1 receptors could represent a novel therapeutic target in chronic inflammatory diseases such as asthma [8].

Small molecular non-peptide orally active antagonists of B1 receptors are desired for clinical drug development for a variety of inflammatory diseases. BI113823 is a small molecule orally active non-peptide B1 receptor antagonist. It exhibits high affinity (Ki) for both the human and rat B1 receptor (5.3 and 13.3 nM, respectively), whereas it has no affinity for the B2 receptor (IC50 > 10,000 nM) [19,20]. As such, BI113823 reduces inflammatory injury in rat and mouse models of sepsis, while exhibiting a favorable cardiovascular profile [19,21].

The aim of the present study, therefore, was to evaluate the anti-inflammatory effects of BI113823 in allergen mediated pulmonary inflammation and to compare these responses with the glucocorticosteroid dexamethasone. To do so, we chose to use a well characterized mouse model of allergen mediated acute pulmonary inflammation, which involves intraperitoneal immunization with OVA precipitated with aluminum hydroxide (alum), followed by repetitive challenges with OVA intratracheally in BALB/c mice [22–24]. We chose this sensitization protocol and mouse strain because in BALB/c mice, as compared to C57BL/6 mice [24,25], systemic immunization with OVA together with alum induces a more profound Th2 mediated lung inflammatory response, which is characterized by a more robust lung eosinophilic response [24,26]. Our results show that treatment with BI113823 starting at 24 h after OVA allergen challenge markedly reduced the allergen mediated pulmonary inflammation.

2. Methods

2.1. Animals

Male BALB/c mice (6–8 weeks of age) were used in all experiments. The mice were housed under controlled light/dark conditions and fed with standard mouse food and water. Animal care and research were approved by the Institutional Animal Care and Use Committee at Chonbuk National University and complied with the Animal Welfare Act.

2.2. OVA-sensitized mouse model of asthma

Male Balb-c mice were randomly assigned to four study groups: (1) sham control, (2) OVA + vehicle, (3) OVA + BI113823 (B1 receptor antagonist), (4) OVA + dexamethasone. Mice were sensitized by giving 75 μg ovalbumin and 2 mg of AL(OH) 3 in phosphate buffered saline intraperitoneally on days 1 and 8. On days 15 to 17, mice were challenged intranasally with 50 μg of ovalbumin. Mice received vehicle (0.1% Natrosol), BI113823 (30 mg/kg, p.o., b.i.d., a gift from Boehringer Ingelheim Pharma KG, Biberach, Germany), and dexamethasone (4 mg/kg, i.p., b.i.d.) on days 16–18.

2.3. Assessment of inflammatory cells in bronchoalveolar lavage (BAL)

On day 19, mice were anesthetized with ketamine (80 mg kg^{-1}, i.m.) plus xylazine (10 mg kg^{-1}, i.m.). Bronchoalveolar lavage (BAL) fluid was collected through a 20-gauge angiocath by instilling 0.5 mL of sterile PBS into the mouse lung and repeated three times. BAL was centrifuged at 1200 rpm for 10 min at 4 °C. The supernatant was stored at −80 °C. The total cell numbers were counted using a hemocytometer. Differential cell counts were determined on the basis of morphologic criteria by staining BAL cell smear with Hemacolor for Microscopy Staining Kit (IVD, Merck, Germany). The dose of the BI11382 used in this study was selected on the basis of preclinical pharmacokinetic and pharmacodynamics studies [19,20]. The dose of the dexamethasone was selected based on its previous use in clinic and laboratory studies showing it to have effective anti-allergic actions [27,28].

2.4. Lung histology

Lung tissues were embedded in paraffin and sections of 5 μm thickness were cut and stained with haematoxylin and eosin. Histological evaluation was determined under light microscope and scored for cellular inflammatory infiltration. Inflammation score was graded by independent pathologist who was not associated with this study. The scoring was made from 0 to 3, higher scores indicate more severe inflammation as previously described [29]. Lung sections were stained with Periodic Acid of Schiff (PAS) for visualizing mucus expression. Mucus scoring was measured using imageJ software.

2.5. Biochemical assay

ELISA kits for mouse tumor necrosis factor (TNF)-α (minimum detectable concentration: 4 pg/mL; R & D Systems, Minneapolis, MN), interleukin 1-beta (IL-1β) (minimum detectable concentration: 2 pg/mL BioLegend, San Diego, CA) and IgE (minimum detectable concentration: 1 ng/mL BD Biosciences, San Jose, CA) were used to determine the concentrations of these mediators in BAL fluids. The protein concentrations of BAL fluids were determined using a Smart BCA Assay Kit (Intron Biotechnology Inc., South Korea). Neutrophil accumulation in the lung was measured by determining myeloperoxidase (MPO) activity as previously described [30].

2.6. Kinin B1 and B2 receptor mRNA expression

Transcript levels of B1 and B2 receptors in lung tissues were assessed by quantitative RT-PCR as previously described [19].

2.7. Immunohistochemistry

Lung sections were deparaffinized, hydrated and incubated in 10 mM sodium citrate buffer at 99 °C for 20 min for antigen retrieval. Sections were incubated with matrix metalloproteinase (MMP)-2, MMP-9, COX-2, iNOS or B1 receptor antibodies (all from Santa Cruz Biotechnology, Santa Cruz, CA) overnight followed by a two hour incubation with FITC-labeled goat anti-rabbit IgG secondary antibody (sc-2012, Santa Cruz Biotechnology) or with Alexa Fluor 594 (Goat pAb to rb IgG alexa Flour 594, abcam). In negative control sections, the primary or secondary antibody was replaced with phosphate buffered saline (PBS) (data not shown). Sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Ultra Cruz Mounting Medium, Santa Cruz Biotechnology) and cover slipped. Fluorescent images were taken by a Nikon Eclipse TE2000-U fluorescence microscope (Nikon Corp., Tokyo, Japan) and a Nikon LWD 0.52 digital camera. Fluorescent intensities were quantified using Image Pro Premier 9.1 software.

2.8. Statistical analysis

All data are reported as mean ± SEM. Statistical differences were determined by analysis of variance for repeated measures followed by Bonferroni's post hoc test using GraphPad Prism 5. P values <0.05 were considered to indicate significant differences.