Allergology International 64 (2015) S46–S53

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Effect of beta2-adrenergic agonists on eosinophil adhesion, superoxide anion generation, and degranulation

Toru Noguchi [a, b, c], Kazuyuki Nakagome [a, b, *], Takehito Kobayashi [a, b, c], Yutaka Ueda [a, d], Tomoyuki Soma [a, b], Hidetomo Nakamoto [c], Makoto Nagata [a, b]

[a] Allergy Center, Saitama Medical University, Saitama, Japan
[b] Department of Respiratory Medicine, Saitama Medical University, Saitama, Japan
[c] Department of General Internal Medicine, Saitama Medical University, Saitama, Japan
[d] Department of Pediatrics, Saitama Medical University, Saitama, Japan

ARTICLE INFO

Article history:
Received 18 January 2015
Received in revised form
17 May 2015
Accepted 20 May 2015
Available online 5 July 2015

Keywords:
Adhesion molecule
Beta2-adrenergic agonist
Chemokine
Cysteinyl leukotrienes
Eosinophils

Abbreviations:
ANOVA, analysis of variance; cAMP, cyclic adenosine 3′,5′-monophosphate; CXCR, CXC chemokine receptor; cysLT, cysteinyl leukotriene; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; FOR, formoterol; HBSS, Hank's balanced salt solution; ICAM, intercellular cell adhesion molecule; IL, interleukin; IP-10, IFN-γ-inducible protein of 10 kDa; O_2^-, superoxide anion; PAF, platelet activating factor; rh, recombinant human; RV, rhinovirus; SAL, salbutamol; SOD, superoxide dismutase; Th, T helper

ABSTRACT

Background: Eosinophils play important roles in the development of asthma exacerbation. Viral infection is a major cause of asthma exacerbation, and the expression of IFN-γ-inducible protein of 10 kDa (IP-10) and cysteinyl leukotrienes (cysLTs) is up-regulated in virus-induced asthma. As β2-adrenergic agonists, such as formoterol or salbutamol, are used to treat asthma exacerbation, we examined whether formoterol or salbutamol could modify eosinophil functions such as adhesiveness, particularly those activated by cysLTs or IP-10.

Methods: Eosinophils were isolated from the blood of healthy subjects and were pre-incubated with either formoterol or salbutamol, and subsequently stimulated with IL-5, LTD_4, or IP-10. Adhesion of eosinophils to intercellular cell adhesion molecule (ICAM)-1 was measured using eosinophil peroxidase assays. The generation of eosinophil superoxide anion (O_2^-) was examined based on the superoxide dismutase-inhibitable reduction of cytochrome C. Eosinophil-derived neurotoxin (EDN) release was evaluated by ELISA as a marker of degranulation.

Results: Neither formoterol nor salbutamol suppressed the spontaneous adhesion of eosinophils to ICAM-1. However, when eosinophils were activated by IL-5, LTD_4, or IP-10, formoterol, but not salbutamol, suppressed the adhesion to ICAM-1. Formoterol also suppressed IL-5, LTD_4, or IP-10 induced eosinophil O_2^- generation or EDN release.

Conclusions: These findings suggest that formoterol, but not salbutamol, suppresses eosinophil functions enhanced by IL-5, LTD_4, or IP-10. As these factors are involved in the development of asthma exacerbation, our results strongly support the hypothesis that administration of formoterol is a novel strategy for treating asthma exacerbation.

Introduction

Eosinophils play important roles in the pathophysiology of asthma through the release of a variety of inflammatory mediators including major basic protein, cysteinyl leukotrienes (cysLTs), reactive oxygen species, and cytokines.[1,2] Eosinophils are also involved in the development of asthma exacerbation. For example, Green *et al.* reported that a treatment strategy directed at normalization of the ratio of eosinophils in induced sputum reduces asthma exacerbation.[3] Among T helper (Th) 2 cytokines, interleukin (IL)-5 is a well-recognized mediator in eosinophilic inflammation,[4] and the *in vitro* effects on eosinophils include prolongation of eosinophil survival and modification of their functions.[5,6] Recent studies suggested that in asthmatics with persistent sputum eosinophilia, treatment with anti-IL-5 mAb reduced asthma exacerbations and the requirement of systemic

* Corresponding author. Department of Respiratory Medicine, Allergy Center, Saitama Medical University, 38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-0495, Japan.
E-mail address: nakagomek-tky@umin.ac.jp (K. Nakagome).
Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2015.05.009

corticosteroids,[7–9] implicating the role of eosinophils or IL-5 in the development of asthma exacerbation.

CysLTs, such as LTC_4, LTD_4, and LTE_4, also contribute to the accumulation of eosinophils in the tissues of asthmatic airways. We have reported that LTD_4 up-regulates the expression of β2 integrins on human eosinophils *in vitro* and augments eosinophil adhesion.[10] We also reported that LTD_4 directly induces transendothelial migration, superoxide anion (O_2^-) generation, and degranulation.[11] In fact, administration of cysLT1 receptor antagonists reduces the number of eosinophils in sputum or blood in asthma patients[12,13] and the frequency of asthma exacerbation in children.[14] Therefore, suppression of IL-5 (or Th2)-mediated or cysLT-mediated eosinophilic inflammation could be a useful strategy for inhibiting asthma exacerbation.

Acute respiratory infections are a major cause of asthma exacerbation.[15,16] The role of IFN-γ-inducible protein of 10 kDa (IP-10) in virus-induced asthma exacerbation has been highlighted.[17–19] We reported that IP-10 up-regulates eosinophil functions such as adhesion and O_2^- generation through CXC chemokine receptor (CXCR) 3 and β2 integrin.[20] We also reported that IP-10 induces degranulation and produces a number of cytokines/chemokines.[20] Therefore, during virus-induced asthma exacerbation IP-10 could directly activate the function of eosinophils in the airway.

β2-adrenoreceptor agonists, such as formoterol and salbutamol, are used to treat asthma exacerbation. Among these drugs, a combination of formoterol and budesonide, especially when used as reliever therapy, suppressed the rate of moderate or severe excerbation,[21–23] suggesting that formoterol may have other functions in addition to its bronchodilating effect. To date, whether β2-adrenoreceptor agonists are capable of suppressing eosinophilic inflammation remains unknown. Some reports suggest that β2-adrenoreceptor agonists suppress the function of mast cells,[24,25] neutrophils,[26–30] eosinophils,[31–33] and T cells,[34] although they are still controversial.[35–38] However, the effect of formoterol or salbutamol on the function of eosinophils, particularly eosinophils activated by IL-5, cysLTs, or IP-10, has not been fully established.

The initial steps of eosinophil accumulation in the asthmatic airway are adhesion to and subsequent transmigration across endothelial cells. Therefore, interaction between eosinophils and adhesion molecules would contribute to the development of airway inflammation in bronchial asthma. In this study, we examined whether formoterol or salbutamol could modify eosinophil functions such as adhesiveness, activated by IL-5, cysLTs, or IP-10. We found that formoterol, but not salbutamol, suppressed eosinophil adhesion to intercellular cell adhesion molecule (ICAM)-1 when eosinophils were stimulated with IL-5, cysLTs, or IP-10. We also found that formoterol suppressed the IL-5, LTD_4, or IP-10-induced eosinophil O_2^- generation or degranulation.

Methods

Preparation of eosinophils

Eosinophils were isolated from peripheral blood collected from non-atopic healthy donors with a peripheral blood differential eosinophil count of <5%. The numbers of males and females, ranging in age from 21 to 59 years, were comparable among the donors. We received approval from the Ethical Committee of Saitama Medical University, and informed consent was obtained before collection of each blood sample. Eosinophils were isolated by negative selection using immunomagnetic beads, as described.[10,11,20,39,40] Over 98% of the cells were eosinophils, as determined by morphologic criteria using May-Grünwald-Giemsa staining. Eosinophil viability was >99%, as determined by Trypan blue dye exclusion. Eosinophils were resuspended in Hank's balanced salt solution (HBSS) supplemented with gelatin to a final concentration of 0.1% (HBSS/gel).

Eosinophil adhesion assay

Eosinophil adhesion to recombinant human (rh)-ICAM-1-coated plates was assessed based on the residual eosinophil peroxidase (EPO) activity of adherent eosinophils, as described.[10,11,20,39,40] Eosinophils were pre-incubated with formoterol (10 nM/100 nM/1000 nM) or salbutamol (10 nM/100 nM/1000 nM) at 37 °C for 15 min. The eosinophils (100 μl of 1×10^5 cells/ml in HBSS/gel) were then incubated with or without IL-5 (100 pM), LTD_4 (100 pM), or IP-10 (100 nM) in rh-ICAM-1-coated plates at 37 °C for 20 min. The plates were washed with HBSS and 100 μl of HBSS/gel was then added to the wells. Standards comprised of 100 μl of serially diluted cell suspensions (1×10^3, 3×10^3, 1×10^4, 3×10^4, and 1×10^5 cells/ml) were added to the empty wells. The EPO substrate (1 mM o-phenylenediamine, 1 mM H_2O_2, and 0.1% Triton X-100 in Tris buffer, pH 8.0) was then added to all wells and the plates were incubated for 30 min at room temperature. The reaction was stopped by adding 20 μl of 4 M H_2SO_4 and absorbance was measured at 490 nm. Each experiment was performed in quadruplicate using eosinophils from a single donor, and the percentage eosinophil adhesion was determined from mean values that were calculated from log dose response curves. Eosinophil viability after incubation was >98%, as determined by Trypan blue dye exclusion.

Eosinophil O_2^- generation

Eosinophil O_2^- generation was measured in 96-well plates (Corning, NY, USA) as described based on the superoxide dismutase (SOD)-inhibitable reduction of cytochrome C.[11,20] We initially added SOD (0.2 mg/ml in HBSS/gel; 20 μl) to SOD control wells and then HBSS/gel to all wells to bring the final volume to 100 μl. The eosinophil density was adjusted to 1.25×10^6 cells/ml of HBSS/gel mixed 4:1 with cytochrome C (12 mg/ml of HBSS/gel), and 100 μl of eosinophil suspension was then added to all wells. After treatment with formoterol and stimulation with IL-5, LTD_4, or IP-10, the absorbance of the cell suspensions in the wells was measured at 550 nm in an Immuno-Mini (NJ-2300; Japan Intermed, Tokyo, Japan), followed by repeated measurements over the next 240 min. The plates were incubated in a 5% CO_2 incubator at 37 °C between measurements. Each reaction was evaluated in duplicate against the control reaction in wells containing 20 μg/ml of SOD. The results were adjusted for a 1-ml reaction volume, and O_2^- generation was calculated at an extinction coefficient of 21.1 mM^{-1} cm^{-1}, as nanomoles of cytochrome C reduced per 1.0×10^6 cells/ml minus the SOD control. The maximum value during the incubation time was examined to evaluate the effects of various factors on eosinophil O_2^- generation. Cell viability, determined by Trypan blue exclusion at the end of each experiment, remained at 95% after a 240-min incubation with the activator.

Eosinophil degranulation

Eosinophils (1×10^6 cells/ml) in 96-well plates were incubated for the 240 min that were required for measurement of O_2^- generation, and were then immediately centrifuged (700 g) at 4 °C for 15 min. Recovered cell-free supernatants were subjected to eosinophil-derived neurotoxin (EDN) analysis, as described previously.[11,20] Levels of EDN were quantified using ELISA kits (Medical and Biological Laboratory, Nagoya, Japan).