



Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

The epidermal growth factor receptor inhibitor AG1478 inhibits eosinophilic inflammation in upper airways

Shino Shimizu ^{*}, Kumiko Takezawa-Yasuoka, Takao Ogawa, Ichiro Tojima, Hideaki Kouzaki, Takeshi Shimizu

Department of Otorhinolaryngology, Shiga University of Medical Science, Otsu, Shiga, Japan

ARTICLE INFO

Article history:

Received 9 June 2017

Received in revised form 23 October 2017

accepted with revision 22 November 2017

Available online xxxx

Keywords:

Eosinophils

Epidermal growth factor receptor

Eotaxin-1

RANTES

GM-CSF

Mucus

ABSTRACT

Mucus hypersecretion and eosinophil infiltration are important characteristics of eosinophilic inflammation in upper airways, such as allergic rhinitis and chronic rhinosinusitis with nasal polyp. EGFR transactivation induces mucus and inflammatory cytokine secretion from airway epithelial cells. However, the roles of EGFR in eosinophilic inflammation in upper airways are still unknown. The purpose of the study is to elucidate the effects of the EGFR inhibitor AG1478 on eosinophilic airway inflammation. AG1478 significantly inhibited thrombin-induced GM-CSF secretion from nasal epithelial cells and thrombin-induced secretion of eotaxin-1 and RANTES from nasal fibroblasts. Intranasal instillation of AG1478 inhibited OVA-induced goblet cell metaplasia, mucus production and eosinophil/neutrophil infiltration in rat nasal epithelium, as did intraperitoneal injection of AG1478. These results indicate that EGFR transactivation plays an important role in eosinophilic airway inflammation. Intranasal instillation of an EGFR inhibitor may be a new therapeutic approach for the treatment of intractable eosinophilic inflammation in upper airways.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Mucus hypersecretion and eosinophil infiltration are important characteristics of eosinophilic airway inflammation such as allergic rhinitis (AR), chronic rhinosinusitis with nasal polyp (CRSwNP) and bronchial asthma [1–3]. A variety of inflammatory mediators and inflammatory cells are involved in the process of eosinophilic airway inflammation, and granulocyte macrophage-colony stimulating factor (GM-CSF), eotaxin-1, regulated upon activation, normal T cell expressed and secreted (RANTES), and vascular endothelial growth factor (VEGF) are important cytokines that stimulate the infiltration, activation and survival of eosinophils [4,5].

Activation of the coagulation system occurs in patients with eosinophilic airway inflammation [6]. We previously demonstrated the presence of thrombin in nasal secretions from patients with allergic rhinitis or CRSwNP [7,8]. Thrombin is an effector enzyme of the coagulation system with important biological functions not only in thrombosis and hemostasis but also in inflammation [9]. We previously reported that nasal epithelial cells and fibroblasts express all four protease-activated receptors (PARs: PAR-1, PAR-2, PAR-3, PAR-4), and that thrombin

stimulates secretion of the MUC5AC mucin, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) from cultured airway epithelial cells via PAR-1 [7,8,10]. We also reported that thrombin and FXa (activated coagulation factor X) stimulate the secretion of eotaxin-1, IL-8, TGF- β and fibronectin from cultured human nasal fibroblasts via PAR-1 or PAR-2 [11]. These results indicate that activation of the coagulation system is implicated in eosinophilic inflammation through the stimulation of mucus hypersecretion and cytokine production by thrombin and FXa.

Eosinophil-epithelial cell interactions play a crucial role in the initiation and development of eosinophilic inflammation. We demonstrated that co-culture of human airway epithelial (NCI-H292) cells and eosinophilic EoL-1 cells or peripheral eosinophils stimulates secretion of the MUC5AC mucin, IL-8, PDGF and VEGF from the epithelial cells [12]. Epidermal growth factor receptor (EGFR) signaling is important for mucin production in response to stimulation of airway epithelial cells by allergens, viruses, neutrophil elastase, and cigarette smoke [13]. We found that an EGFR tyrosine kinase inhibitor suppressed secretion not only of the MUC5AC mucin, but also of IL-8, PDGF and VEGF that was induced by eosinophil-epithelial cell interactions, and that metalloproteinase-mediated EGFR transactivation of epithelial cells is involved in this process [12].

The EGFR is expressed in the epithelial cells of upper and lower airways, and it is up-regulated in allergic rhinitis, CRSwNP and bronchial asthma [14–16]. Thrombin-induced secretion of the MUC5AC mucin from cultured airway epithelial cells was significantly inhibited by the EGFR inhibitor AG1478. In our previous study, we evaluated the effects

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyp; EGFR, epidermal growth factor receptor; GM-CSF, granulocyte macrophage-colony stimulating factor; OVA, ovalbumin; RANTES, regulated upon activation, normal T cell expressed and secreted.

^{*} Corresponding author at: Department of Otorhinolaryngology, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520-2192, Japan.

E-mail address: sshino@belle.shiga-med.ac.jp (S. Shimizu).

of AG1478 using an in vivo rat model with LPS-induced nasal inflammation, and found that intranasal instillation of AG1478 significantly inhibited LPS-induced goblet cell metaplasia, mucus production and neutrophil infiltration in rat nasal mucosa, as did intraperitoneal injection of AG1478 [17].

In the present study, to elucidate the effects of the EGFR tyrosine kinase inhibitor AG1478 on mucus hypersecretion and eosinophil infiltration in eosinophilic airway inflammation, we examined (1) the in vitro effects of AG1478 on thrombin-induced secretion of GM-CSF, eotaxin-1 and RANTES from cultured human nasal epithelial cells or fibroblasts, and (2) the in vivo effects of AG1478 on ovalbumin (OVA)-induced goblet cell metaplasia, mucus hypersecretion and eosinophil infiltration in the nasal mucosa of OVA-sensitized rats. The inhibitory effects of intraperitoneal injection and intranasal instillation of AG1478 were examined and compared.

2. Materials and methods

2.1. Cell culture and determination of GM-CSF, eotaxin-1 and RANTES

Nasal polyps were obtained during endoscopic surgery from three patients with CRSwNP. Informed consent was obtained from all subjects before sampling. The clinical protocol was approved by the Shiga University of Medical Science Institutional Review Board for Clinical Investigation. Nasal epithelial cells were scratched from nasal polyps using dedicated curettes (Panasonic, Ehime, Japan). Nasal epithelial cells were suspended in supplemented basal epithelial growth medium (BEGM; Lonza Corp., Walkersville, MD, USA) and cultured in a type I collagen-coated 25-cm² flask (Iwaki, Tokyo, Japan) in a humidified incubator at 37 °C with 5% CO₂. Once confluent, nasal epithelial cells were subcultured and seeded in type I collagen-coated 24-well tissue culture plates (Iwaki).

Submucosal tissues of nasal polyps were cut into small pieces, and nasal polyp fibroblasts were expanded in 75-cm² flasks in Dulbecco's modified Eagle's medium (DMEM, WAKO, Tokyo, Japan) supplemented with 10% FBS, 100 µg/ml penicillin, and 100 µg/ml streptomycin, in a humidified incubator at 37 °C with 5% CO₂. Once confluent, fibroblasts were subcultured and seeded onto 24-well tissue culture plates.

After incubation of the confluent cells (epithelial cells or fibroblasts) for 18 h in basal medium without supplements, thrombin (Nacalai Tesque, Tokyo, Japan) and/or AG1478 were added and the cells were incubated for an additional 24 h. Supernatants were collected and stored at –20 °C until use. The concentrations of GM-CSF, eotaxin-1, and RANTES in cell supernatants were determined using DuoSet immunoassay kits (R&D Systems, Minneapolis, MN, USA), following the manufacturer's protocol.

2.2. Mucus hypersecretion and eosinophil infiltration in rat nasal epithelium

The experimental protocol was approved by the Committee for Animal Care and Ethics of Shiga University of Medical Science and it was carried out following the guidelines of the National Institute of Health. Sensitization and challenge of rats were performed as previously described [18]. Specific pathogen-free male Fisher-344 rats at 6 weeks of age were used in this study. Rats were sensitized with an intraperitoneal injection of 1 ml of saline solution containing 200 µg of OVA (grade V; Sigma-Aldrich Corp., St Louis, MO, USA) and 5 mg of aluminum hydroxide (Al(OH)₃) on days 1, 2, 3, and 11. On days 19, 20 and 21, rats were anesthetized with isoflurane and intranasally challenged with OVA by instillation of 0.1 ml of saline solution containing 10 mg of OVA into both airways of the nasal cavity once per day. On day 22, rats were anesthetized with isoflurane and sacrificed. Sham-challenged rats received 0.1 ml saline solution in the same manner. AG1478 (1 or 10 mg/kg) dissolved in 0.3 ml dimethyl sulfoxide (DMSO) was intraperitoneally injected at 1 h before the intranasal challenge with OVA, or AG1478 (1

or 10 mg/kg) dissolved in 0.05 ml DMSO was intranasally instilled at 1 h after the intranasal challenge with OVA for 3 consecutive days (Figs. 3A and 4A).

The rats were painlessly killed with an intraperitoneal overdose of sodium pentobarbital at 24 h after the last intranasal OVA instillation. The head of each rat was removed, fixed in 10% neutral buffered formalin for 72 h, and then decalcified in 5% trichloroacetic acid for 7 days. The nasal cavity was transversely sectioned at the level of the incisive papilla, and the tissue block was embedded in paraffin. Tissue sections 4 µm-thick were stained with Alcian blue-periodic acid-Schiff (AB-PAS) and hematoxylin. The percentage area of AB-PAS-stained mucosubstance in the epithelial surface was determined by an image analyzer in which a region of nasal epithelium over 2 mm in width (1 mm each side of the nasal septum) at the center of the septal cartilage. The infiltrating eosinophils and neutrophils in nasal mucosa were examined by hematoxylin-eosin staining. The numbers of eosinophils and neutrophils in nasal septal mucosa were counted over both sides of the nasal septal cartilage under a microscope using an oil immersion objective lens (magnification: ×1000). All measurements were done by one blinded observer.

2.3. Statistical analysis

All data are expressed as means and standard deviation (SD). The significance of differences between variables was assessed by analysis of variance with post hoc analysis using the Tukey-Kramer test or the Steel-Dwass test. Statistical analyses were carried out using the StatView 5.0 software package (Abacus Concepts, Berkeley, CA, USA). *P* values < 0.05 were considered to indicate a significant difference.

3. Results

3.1. In vitro effects of AG1478 on the secretion of GM-CSF from nasal epithelial cells

Cultured nasal epithelial cells were incubated with thrombin (0.01, 0.1, or 1 U/ml), FXa (0.01, 0.1, or 1 U/ml) or PAR agonist peptides for 24 h, following which the level of GM-CSF secreted into the cell supernatant was examined. Thrombin and FXa dose-dependently stimulated GM-CSF secretion from cultured nasal epithelial cells (Fig. 1A and B). PAR-1 and PAR-2 agonist peptides also significantly stimulated GM-CSF secretion whereas PAR-3 and PAR-4 agonist peptides showed no effect (Fig. 1C). AG1478 (0.1 or 1 µM) dose-dependently inhibited GM-CSF secretion from both of unstimulated and thrombin (1 U/ml)-stimulated nasal epithelial cells (Fig. 2A).

3.2. In vitro effects of AG1478 on the secretion of eotaxin-1 and RANTES from nasal fibroblasts

Thrombin (1 U/ml) also significantly stimulated the secretion of eotaxin-1 and RANTES from cultured nasal fibroblasts. AG1478 (10 µM) significantly inhibited eotaxin-1 secretion from unstimulated nasal fibroblasts (Fig. 2B). And AG1478 (1 or 10 µM) dose-dependently inhibited eotaxin-1 and RANTES secretion from thrombin (1 U/ml)-stimulated nasal fibroblasts (Fig. 2B and C).

3.3. In vivo effects of AG1478 on mucus production and eosinophil infiltration

We first examined the effects of intraperitoneal injection of AG1478 on OVA-induced changes when AG1478 was injected one hour prior to intranasal OVA challenge in OVA-sensitized rats (Fig. 3A). Intranasal instillation of OVA for 3 consecutive days into OVA-sensitized rats at 1 h after injection of control DMSO significantly induced hypertrophic and metaplastic changes of goblet cells in the nasal septal epithelium. A few goblet cells were observed in the control groups of intranasal instillations of saline instead of OVA at 1 h after intraperitoneal injection of

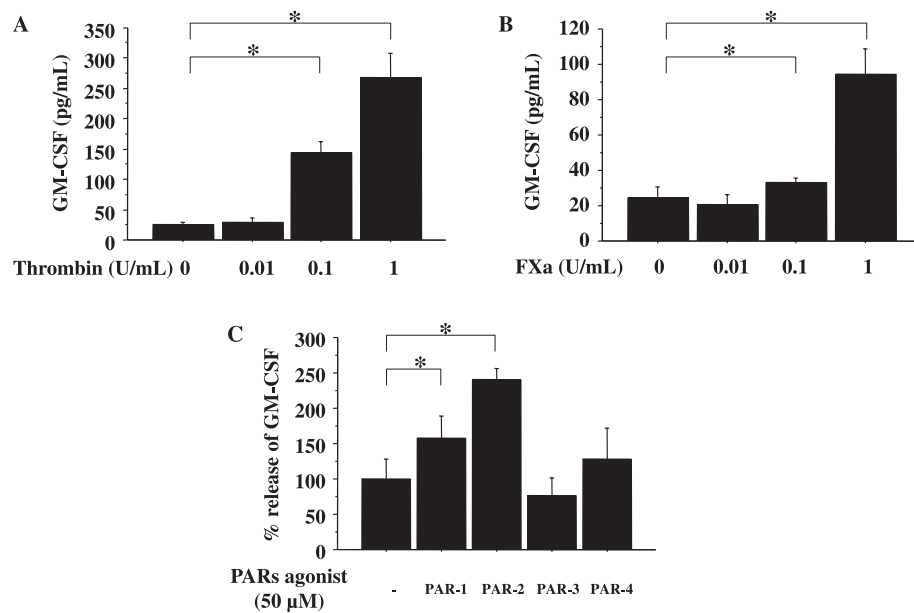


Fig. 1. Effects of thrombin (A), FXa (B) and the PAR agonist peptides PAR-1, PAR-2, PAR-3, and PAR-4 (C) on the secretion of GM-CSF from cultured nasal epithelial cells ($n = 6$). The effects of PAR agonist peptides are shown as percent secretion of GM-CSF relative to control. Thrombin, FXa, and PAR-1 and PAR-2 agonists significantly stimulated the secretion of GM-CSF; $*P < 0.05$, analysis of variance with post hoc analysis using the Tukey-Kramer test.

either DMSO or AG1478. Intraperitoneal injection of AG1478 (1 or 10 mg/kg) 1 h before OVA challenge dose-dependently inhibited OVA-induced goblet cell metaplasia and mucus production in rat nasal epithelium (Fig. 3A, B and C). Such intraperitoneal injection of AG1478, but not of DMSO, also significantly and dose-dependently suppressed OVA-induced infiltration of eosinophils and neutrophils into rat nasal epithelium (Fig. 3D and E).

We next examined the effects of intranasal instillation of AG1478 on OVA-induced changes when AG1478 was administered one hour post intranasal OVA challenge in OVA-sensitized rats (Fig. 4A). A few goblet cells were observed in the control groups of intranasal instillation of saline 1 h before intranasal instillation of DMSO or AG1478. Intranasal

instillation of AG1478 (1 or 10 mg/kg) one hour after intranasal instillation of OVA dose-dependently inhibited OVA-induced goblet cell metaplasia and mucus production in rat nasal epithelium whereas DMSO instillation had no effect (Fig. 4B and C). Such intranasal instillation with AG1478, but not with DMSO, also significantly and dose-dependently suppressed OVA-induced infiltration of eosinophils and neutrophils into rat nasal epithelium (Fig. 4D and E).

4. Discussion

Allergic airway inflammation is associated with increased vascular permeability and leakage of plasma coagulation factors, leading to

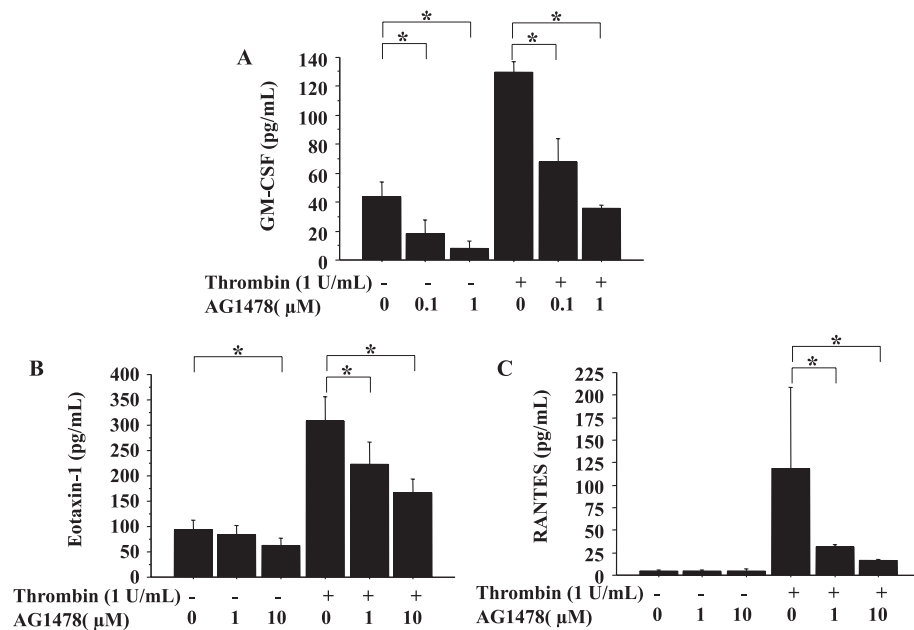


Fig. 2. Effects of the EGFR inhibitor, AG1478, on the thrombin-induced secretion of GM-CSF from nasal epithelial cells (A), and of eotaxin-1 (B) and RANTES (C) from nasal fibroblasts ($n = 6$). AG1478 (0.1 or 1 μM) dose-dependently inhibited thrombin (1 U/ml)-induced secretion of GM-CSF from nasal epithelial cells and of eotaxin-1 and RANTES from nasal fibroblasts. $*P < 0.05$, analysis of variance with post hoc analysis using the Tukey-Kramer test.

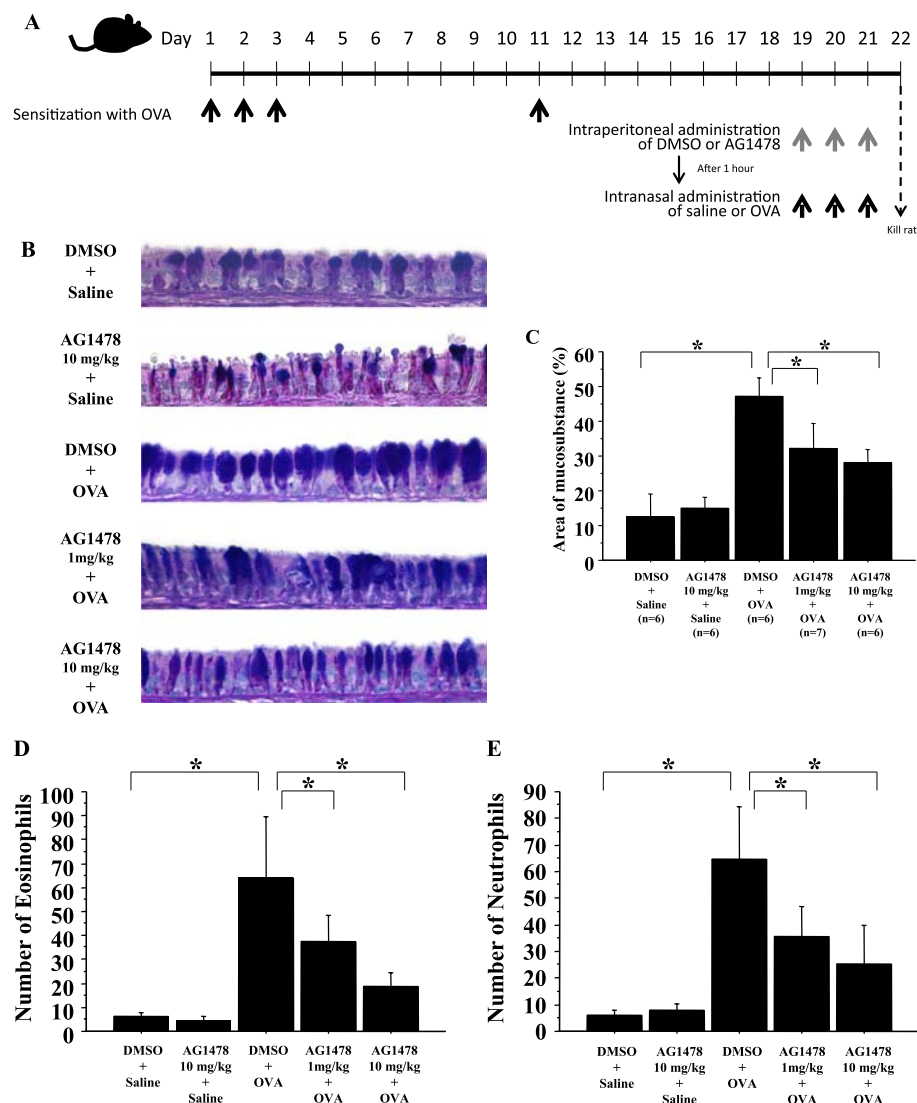


Fig. 3. Effects of intraperitoneal injection of AG1478 on OVA-induced changes in the nasal epithelium of OVA-sensitized rats. (A) Timeline of intraperitoneal sensitization and intranasal challenge with OVA or control saline. AG1478 or control DMSO was intraperitoneally injected at 1 h before OVA challenge. The effects of AG1478 injection on goblet cell metaplasia (Alcian blue-periodic acid-Schiff) (B), mucus production (C), eosinophil infiltration (D), and neutrophil infiltration (E) in rat nasal epithelium were measured. Intraperitoneal injection of AG1478 (1 or 10 mg/kg) significantly and dose-dependently inhibited OVA-induced goblet cell metaplasia, mucus production and infiltration of eosinophils and neutrophils. * $P < 0.05$, the Steel-Dwass test.

activation of the coagulation system in the extravascular space [19,20]. Tissue factor (TF) is an important initial upstream protein in the extrinsic coagulation cascade and the most potent stimulator of this cascade. Leakage of coagulation factors into the tissues induces factor VIIa to bind to TF on the cell surface, and the resulting complex binds to factor X, converting it to the activated form FXa, ultimately leading to thrombin formation and fibrin deposition [21]. The coagulation system is activated in allergic inflammation of upper airways. We previously found significant concentrations of thrombin in nasal secretions from patients with AR or CRSwNP, and that thrombin was significantly increased after provocation with a disc of the house dust mite in AR patients [7,8]. We also previously found that infiltrating eosinophils expressed TF, and that TNF- α or thrombin enhanced the TF activity of epithelial cells [7,22].

Coagulation factors such as thrombin and FXa play important roles not only in thrombosis and homeostasis, but also in inflammation through interactions with PARs that are expressed on epithelial cells and fibroblasts [23]. PAR-1 is a major receptor for thrombin, and PAR-2 for FXa. Thrombin stimulates the secretion of the MUC5AC mucin and profibrotic cytokines such as PDGF and VEGF from nasal epithelial cells via PAR-1 [7,8,10]. Thrombin also stimulates the chemotaxis of

human eosinophils via PAR-1 [24]. GM-CSF, eotaxin-1 and RANTES are important cytokines for the infiltration, activation and survival of eosinophils [4]. Cultured nasal epithelial cells were shown to produce significant amount of GM-CSF in patients with AR or CRSwNP [25]. Higher concentrations of GM-CSF in nasal lavage fluids have been reported in patients with chronic rhinitis or aspirin-exacerbated respiratory disease [26,27]. In the present study, thrombin and FXa stimulated the secretion of GM-CSF from cultured nasal epithelial cells via PAR-1 or PAR-2. Nasal fibroblasts are major sources of eotaxin-1 and RANTES in allergic inflammation [28,29]. We previously found that cultured nasal fibroblast produced eotaxin-1, and that thrombin and FXa stimulated eotaxin-1 production via PAR-1 or PAR-2 [11]. These results indicate that airway inflammation activates the coagulation system, and that PARs-mediated responses provide a direct link between coagulation and allergic inflammation.

In the present study, the EGFR tyrosine kinase inhibitor AG1478 significantly inhibited thrombin-induced secretion of GM-CSF from cultured nasal epithelial cells and thrombin-induced secretion of eotaxin-1 and RANTES from cultured nasal fibroblasts. These results indicate that EGFR transactivation in both epithelial cells and fibroblasts is

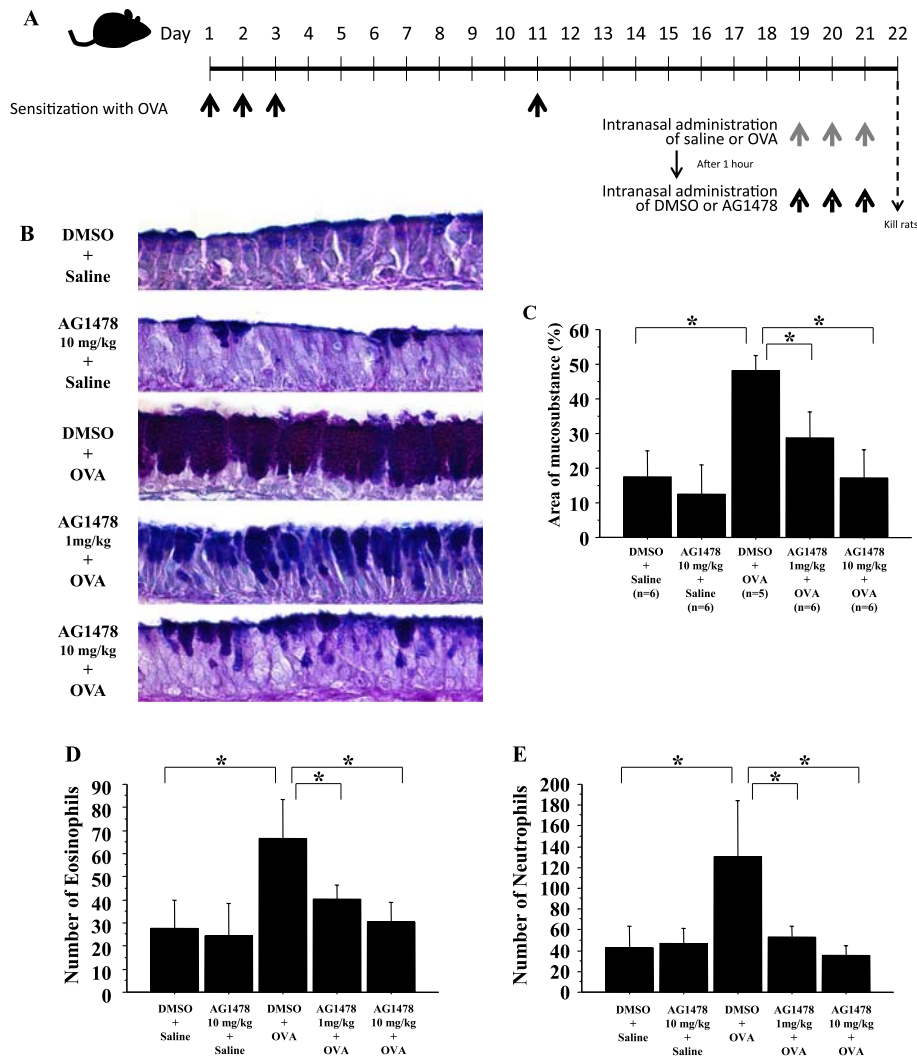


Fig. 4. Effects of intranasal instillation with AG1478 on OVA-induced changes in the nasal epithelium of OVA-sensitized rats. (A) Timeline of intraperitoneal sensitization and intranasal challenge with OVA. AG1478 or control DMSO was intranasally instilled at 1 h after OVA or control saline challenge. The effects of AG1478 on goblet cell metaplasia (Alcian blue-periodic acid-Schiff) (B), mucus production (C), eosinophil infiltration (D), and neutrophil infiltration (E) in rat nasal epithelium were measured. Intranasal instillation of AG1478 (1 or 10 mg/kg) significantly and dose-dependently inhibited OVA-induced goblet cell metaplasia, mucus production and infiltration of eosinophils and neutrophils. * $P < 0.05$, the Steel-Dwass test.

crucial for thrombin-induced secretion of GM-CSF, eotaxin-1 and RANTES. The EGFR ligand family participates in various cellular functions, such as proliferation, migration, angiogenesis, differentiation, and survival [14,30], and EGFR expression is upregulated in the nasal mucosa of allergic rhinitis or CRSwNP [15,16,31]. We previously reported that thrombin enhanced the mRNA expression of the EGFR in cultured nasal epithelial cells [7]. EGFR signaling is transactivated by various extracellular stimuli such as lipid mediators, toll-like receptor ligands, reactive oxygen species, and PAR ligands [32]. We previously demonstrated that eosinophil-epithelial cell interactions assayed by the co-culture of epithelial cells with eosinophils stimulated secretion of the MUC5AC mucin, PDGF, VEGF, and IL-8 from epithelial cells, and that the EGFR inhibitor AG1478 suppressed these secretions of the MUC5AC mucin and cytokines [12]. A principle mechanism of EGFR transactivation in epithelial cells involves activation of matrix metalloproteinases of the ADAM (a disintegrin and metalloproteinase) family. Ligand-shedding dependent transactivation of the EGFR is mediated by ligands such as amphiregulin and TGF- α , which are cleaved from their membrane-anchored forms by activated metalloproteinase. Although important roles of EGFR signaling have been reported in bronchial and alveolar fibroblasts [33,34], this is the first report to show inhibitory effects of the EGFR inhibitor AG1478 on cytokine secretion from nasal fibroblasts.

Mucus hypersecretion and eosinophil infiltration are common characteristics of allergic airway inflammation [3]. In the present study, hyperplastic and metaplastic changes of goblet cells and eosinophil infiltration were induced by intranasal instillation of OVA into the nasal epithelium of OVA-sensitized rats. Intraperitoneal injection of the EGFR inhibitor AG1478 1 h before the intranasal instillation of OVA significantly inhibited antigen-induced goblet cell metaplasia, mucus production and the infiltration of eosinophils and neutrophils. In previous studies, intratracheal instillation of AG1478 suppressed goblet cell metaplasia in OVA-induced allergic inflammation of rat lung [35], and oral administration of the EGFR inhibitor gefitinib attenuated eosinophil infiltration and airway hyperresponsiveness in OVA-induced allergic inflammation of mouse lung [36]. These results indicate that EGFR inhibitors may have a new therapeutic potential for the treatment of intractable eosinophilic inflammation such as nasal polyposis and bronchial asthma.

In the present study, intranasal instillation of AG1478 1 h after intranasal administration of OVA also significantly inhibited antigen-induced goblet cell metaplasia, mucus production and infiltration of eosinophils and neutrophils in rat nasal epithelium. The inhibitory effects of AG1478 were equivalent whether it was administered by intranasal instillation or intraperitoneal injection. The EGFR tyrosine kinase inhibitors,

gefitinib, erlotinib and afatinib are clinically-used anticancer drugs that have systemic adverse effects such as dermatitis, mucositis, diarrhea, gastrointestinal bleeding and intestinal pneumonia [37]. Intranasal local instillation of an EGFR inhibitor will be a useful way for drug administration because it may avoid such systemic adverse effects.

Author's contributions

SS contributed to study design, research, data collection and wrote the manuscript. KY, TK, and IT contributed data collection. TS designed the study and wrote the manuscript.

Conflict of interest

We have no actual or potential conflict of interest in relation to this paper.

Acknowledgements

We thank the staff of the Central Research Laboratory of Shiga University of Medical Science for their important contributions to the completion of this work. This study was supported by GSK Japan Grant Number 14B003 and JSPS KAKENHI Grant Numbers 15K20200, 16K11212, 26462581.

References

- [1] R. Pawankar, M. Nonaka, Inflammatory mechanisms and remodeling in chronic rhinosinusitis and nasal polyps, *Curr. Allergy Asthma Rep.* 7 (2007) 202–208.
- [2] S. Al-Muhsen, J.R. Johnson, Q. Hamid, Remodeling in asthma, *J. Allergy Clin. Immunol.* 128 (2011) 451–462 (quiz 463–454).
- [3] B. Sin, A. Togias, Pathophysiology of allergic and nonallergic rhinitis, *Proc. Am. Thorac. Soc.* 8 (2011) 106–114.
- [4] M. Wegmann, Targeting eosinophil biology in asthma therapy, *Am. J. Respir. Cell Mol. Biol.* 45 (2011) 667–674.
- [5] G. Ciprandi, G. Murdaca, B.M. Colombo, M. De Amici, G.L. Marseglia, Serum vascular endothelial growth factor in allergic rhinitis and systemic lupus erythematosus, *Hum. Immunol.* 69 (2008) 510–512.
- [6] E. Gabazza, O. Taguchi, S. Tamaki, H. Takeya, H. Kobayashi, H. Yasui, T. Kobayashi, O. Hataji, H. Urano, H. Zhou, K. Suzuki, Y. Adachi, Thrombin in the airways of asthmatic patients, *Lung* 177 (1999) 253–262.
- [7] S. Shimizu, T. Shimizu, J. Morser, T. Kobayashi, A. Yamaguchi, L. Qin, M. Toda, C. D'Alessandro-Gabazza, T. Maruyama, T. Takagi, Y. Yano, Y. Sumida, T. Hayashi, Y. Takei, O. Taguchi, K. Suzuki, E.C. Gabazza, Role of the coagulation system in allergic inflammation in the upper airways, *Clin. Immunol.* 129 (2008) 365–371.
- [8] S. Shimizu, E.C. Gabazza, T. Ogawa, I. Tojima, E. Hoshi, H. Kouzaki, T. Shimizu, Role of thrombin in chronic rhinosinusitis-associated tissue remodeling, *Am. J. Rhinol. Allergy* 25 (2011) 7–11.
- [9] E. Gabazza, O. Taguchi, H. Kamada, T. Hayashi, Y. Adachi, K. Suzuki, Progress in the understanding of protease-activated receptors, *Int. J. Hematol.* 79 (2004) 117–122.
- [10] S. Shimizu, E. Gabazza, T. Hayashi, M. Ido, Y. Adachi, K. Suzuki, Thrombin stimulates the expression of PDGF in lung epithelial cells, *Am. J. Phys. Lung Cell. Mol. Phys.* 279 (2000) L503–510.
- [11] S. Shimizu, I. Tojima, K. Takezawa, K. Matsumoto, H. Kouzaki, T. Shimizu, Thrombin and activated coagulation factor X stimulate the release of cytokines and fibronectin from nasal polyp fibroblasts via protease-activated receptors, *Am. J. Rhinol. Allergy* 31 (2017) 13–18.
- [12] S. Shimizu, H. Kouzaki, T. Ogawa, K. Takezawa, I. Tojima, T. Shimizu, Eosinophil-epithelial cell interactions stimulate the production of MUC5AC mucin and profibrotic cytokines involved in airway tissue remodeling, *Am. J. Rhinol. Allergy* 28 (2014) 103–109.
- [13] P.R. Burgel, J.A. Nadel, Epidermal growth factor receptor-mediated innate immune responses and their roles in airway diseases, *Eur. Respir. J.* 32 (2008) 1068–1081.
- [14] R. Polosa, G. Prosperini, V. Tomaselli, P.H. Howarth, S.T. Holgate, D.E. Davies, Expression of c-erbB receptors and ligands in human nasal epithelium, *J. Allergy Clin. Immunol.* 106 (2000) 1124–1131.
- [15] M. Amishima, M. Munakata, Y. Nasuhara, A. Sato, T. Takahashi, Y. Homma, Y. Kawakami, Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway, *Am. J. Respir. Crit. Care Med.* 157 (1998) 1907–1912.
- [16] E. Matovinovic, O. Solberg, D. Shusterman, Epidermal growth factor receptor - but not histamine receptor - is upregulated in seasonal allergic rhinitis, *Allergy* 58 (2003) 472–475.
- [17] K. Takezawa, T. Ogawa, S. Shimizu, T. Shimizu, Epidermal growth factor receptor inhibitor AG1478 inhibits mucus hypersecretion in airway epithelium, *Am. J. Rhinol. Allergy* 30 (2016) 1–6.
- [18] T. Shimizu, S. Shimizu, R. Hattori, E.C. Gabazza, Y. Majima, In vivo and in vitro effects of macrolide antibiotics on mucus secretion in airway epithelial cells, *Am. J. Respir. Crit. Care Med.* 168 (2003) 581–587.
- [19] L. Greiff, M. Andersson, J. Erjefält, C. Persson, P. Wollmer, Airway microvascular extravasation and luminal entry of plasma, *Clin. Physiol. Funct. Imaging* 23 (2003) 301–306.
- [20] M. Matthay, J. Clements, Coagulation-dependent mechanisms and asthma, *J. Clin. Invest.* 114 (2004) 20–23.
- [21] K. Mann, Thrombin formation, *Chest* 124 (2003) 4S–10S.
- [22] S. Shimizu, T. Ogawa, K. Takezawa, I. Tojima, H. Kouzaki, T. Shimizu, Tissue factor and tissue factor pathway inhibitor in nasal mucosa and nasal secretions of chronic rhinosinusitis with nasal polyp, *Am. J. Rhinol. Allergy* 29 (2015) 235–242.
- [23] C. Reed, H. Kita, The role of protease activation of inflammation in allergic respiratory diseases, *J. Allergy Clin. Immunol.* 114 (2004) 997–1008 (quiz 1009).
- [24] C. Feistritz, B. Mosheimer, N. Kaneider, M. Riewald, J. Patsch, C. Wiedermann, Thrombin affects eosinophil migration via protease-activated receptor-1, *Int. Arch. Allergy Immunol.* 135 (2004) 12–16.
- [25] T. Ohtoshi, C. Vancheri, G. Cox, J. Gauldie, J. Dolovich, J.A. Denburg, M. Jordana, Monocyte-macrophage differentiation induced by human upper airway epithelial cells, *Am. J. Respir. Cell Mol. Biol.* 4 (1991) 255–263.
- [26] A. Peric, C. Spadijer-Mirkovic, S. Matkovic-Jozin, L. Jovancevic, D. Vojvodic, Granulocyte-macrophage colony-stimulating factor production and tissue eosinophilia in chronic rhinitis, *Int. Arch. Otorhinolaryngol.* 20 (2016) 364–369.
- [27] W.W. Stevens, C.J. Ocampo, S. Berdnikovs, M. Sakashita, M. Mahdavinia, L. Suh, T. Takabayashi, J.E. Norton, K.E. Hulse, D.B. Conley, R.K. Chandra, B.K. Tan, A.T. Peters, L.C. Grammer, A. Kato, K.E. Harris, R.G. Carter, S. Fujieda, R.C. Kern, R.P. Schleimer, Cytokines in chronic rhinosinusitis. Role in eosinophilia and aspirin-exacerbated respiratory disease, *Am. J. Respir. Crit. Care Med.* 192 (2015) 682–694.
- [28] M. Nonaka, R. Pawankar, F. Saji, T. Yagi, Eotaxin synthesis by nasal polyp fibroblasts, *Acta Otolaryngol.* 119 (1999) 816–820.
- [29] K. Yoshifuku, S. Matsune, J. Ohori, Y. Sagara, T. Fukuiwa, Y. Kuroki, IL-4 and TNF- α increased the secretion of eotaxin from cultured fibroblasts of nasal polyps with eosinophil infiltration, *Rhinology* 45 (2007) 235–241.
- [30] R. Polosa, G. Prosperini, S.H. Leir, S.T. Holgate, P.M. Lackie, D.E. Davies, Expression of c-erbB receptors and ligands in human bronchial mucosa, *Am. J. Respir. Cell Mol. Biol.* 20 (1999) 914–923.
- [31] G.Q. Ding, C.Q. Zheng, S.S. Bagga, Up-regulation of the mucosal epidermal growth factor receptor gene in chronic rhinosinusitis and nasal polyposis, *Arch. Otolaryngol. Head Neck Surg.* 133 (2007) 1097–1103.
- [32] H. Daub, F.U. Weiss, C. Wallasch, A. Ullrich, Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors, *Nature* 379 (1996) 557–560.
- [33] H. Yoshie, J. Kirkham-Brown, E. Healy, S.T. Holgate, A.P. Sampson, D.E. Davies, Cysteinyl leukotrienes synergize with growth factors to induce proliferation of human bronchial fibroblasts, *J. Allergy Clin. Immunol.* 119 (2007) 132–140.
- [34] H. Li, X. Yuan, J. Tang, Y. Zhang, Lipopolysaccharide disrupts the directional persistence of alveolar myofibroblast migration through EGF receptor, *Am. J. Phys. Lung Cell. Mol. Phys.* 302 (2012) L569–579.
- [35] K. Takeyama, J. Tamaoki, M. Kondo, K. Isono, A. Nagai, Role of epidermal growth factor receptor in maintaining airway goblet cell hyperplasia in rats sensitized to allergen, *Clin. Exp. Allergy* 38 (2008) 857–865.
- [36] G.Y. Hur, S.Y. Lee, S.H. Lee, S.J. Kim, K.J. Lee, J.Y. Jung, E.J. Lee, E.H. Kang, K.H. Jung, J.H. Kim, C. Shin, J.J. Shim, K.H. In, K.H. Kang, S.H. Yoo, Potential use of an anticancer drug gefitinib, an EGFR inhibitor, on allergic airway inflammation, *Exp. Mol. Med.* 39 (2007) 367–375.
- [37] D.M. Keefe, E.H. Bateman, Tumor control versus adverse events with targeted anti-cancer therapies, *Nat. Rev. Clin. Oncol.* 9 (2011) 98–109.