

Peroxisome proliferator activated receptor γ modulates reactive oxygen species generation and activation of nuclear factor- κ B and hypoxia-inducible factor 1 α in allergic airway disease of mice

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Background: Reactive oxygen species (ROSs) play a crucial role in the pathogenesis of airway inflammation. Peroxisome proliferator activated receptor (PPAR)- γ is also involved in airway inflammation. We have demonstrated that the administration of PPAR γ agonists or adenovirus carrying PPAR γ cDNA (AdPPAR γ) reduced bronchial inflammation and airway hyperresponsiveness. However, the effects of PPAR γ on ROS generation in conditions associated with airway inflammation have not been clarified.

Objective: This study aimed to investigate the effects of the PPAR γ on ROS generation in allergic airway disease of mice.

Methods: We have used a female C57BL/6 mouse model for allergic airway disease to determine the role of PPAR γ .

Results: In this study with an ovalbumin-induced murine model of allergic airway disease, the increased ROS generation and the increased expression of T_H2 cell cytokines, adhesion molecules, chemokines, and vascular endothelial growth factor in lungs after ovalbumin inhalation were significantly reduced by the administration of PPAR γ agonists or AdPPAR γ . We also showed that the increased nuclear factor- κ B and hypoxia-inducible factor 1 α levels in nuclear protein extracts of lung tissues after ovalbumin inhalation were

decreased by the administration of PPAR γ agonists or AdPPAR γ .

Conclusion: These results indicate that the effects of PPAR γ are mediated by the modulation of ROS generation and activation of redox-sensitive transcription factor nuclear factor- κ B and HIF-1 α in allergic airway disease of mice.

Clinical implications: Thus, these findings provide a pivotal molecular mechanism for the use of PPAR γ agonists to prevent and/or treat asthma and other airway inflammatory disorders. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, HIF-1 α , NF- κ B, oxidative stress, peroxisome proliferator activated receptor γ

Asthma is a chronic inflammatory disorder of the airways in which many cell types play a role.¹ Oxidative stress is caused by a large variety of free oxygen radicals known as reactive oxygen species (ROSs). ROSs play a crucial role in the pathogenesis of airway inflammation.² The inflammatory cells recruited to the asthmatic airways have a capability of producing ROSs. Recently, we have demonstrated that oxidative stress is one of the important determinants of asthma and that antioxidant treatment may be a recommendable therapeutic strategy.³

Peroxisome proliferator activated receptors (PPARs) are transcriptional factors belonging to the ligand-activated nuclear receptor superfamily.⁴ They are ubiquitously expressed throughout the body. Three major types have been identified: PPAR α , PPAR β/δ , and PPAR γ . PPAR γ is originally known to regulate adipocyte differentiation and lipid metabolism.⁵ However, accumulating evidence indicates that PPAR γ affects cell cycle, differentiation, and apoptosis.⁶ In addition, PPAR γ activation downregulates synthesis and release of immunomodulatory cytokines from various cell types.^{7,8} Previous studies have shown that PPAR γ is involved in airway inflammation and remodeling in asthma.^{9,10} PPAR γ expression is increased in the airway mucosa of patients with asthma compared with healthy subjects.⁹ Activation of PPAR γ reduces expression of various cytokines, airway

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Supported by grants from the National Research Laboratory Program and from the Medical Science and Engineering Research Center of the Korea Science and Engineering Foundation (R13-2002-038-01004-0), Republic of Korea. Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication November 2, 2005; revised March 16, 2006; accepted for publication March 20, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.03.021

Abbreviations used

AdPPAR γ : Adenovirus carrying peroxisome proliferator activated receptor γ cDNA
 BAL: Bronchoalveolar lavage
 EBD: Evans blue dye
 HIF: Hypoxia-inducible factor
 ICAM-1: Intercellular adhesion molecule 1
 NF- κ B: Nuclear factor- κ B
 PPAR: Peroxisome proliferator activated receptor
 ROS: Reactive oxygen species
 VCAM-1: Vascular cell adhesion molecule 1
 VEGF: Vascular endothelial growth factor

hyperresponsiveness, and activation of eosinophils, which are increased by induction of asthma.¹¹

Studies have demonstrated that PPAR γ agonists have a protective role against oxidative stress. PPAR γ agonists reduced the lesions associated with ischemia-reperfusion of the stomach, kidney, heart, and gut possibly as a result of anti-inflammatory properties.¹²⁻¹⁴ However, the effects of PPAR γ on oxidative stress in conditions associated with airway inflammation have not been clarified. This study investigates the effects of the PPAR γ agonists, rosiglitazone or pioglitazone, or adenovirus gene transfer vector expressing a PPAR γ cDNA (AdPPAR γ) on ROS generation in a murine model of allergic airway disease. We found evidence that the effects of PPAR γ are mediated by the modulation of ROS generation and activation of redox-sensitive transcription factor nuclear factor- κ B (NF- κ B) and hypoxia-inducible factor (HIF)-1 α in allergic airway disease of mice.

METHODS

More detailed information about the [Methods](#) is provided in this article's Online Repository at www.jacionline.org.

Animals and experimental protocol

Female C57BL/6 mice, 8 to 10 weeks of age and free of murine-specific pathogens, were obtained from the Korean Research Institute of Chemistry Technology (Daejeon, Korea). All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School. Standard guidelines for laboratory animal care were followed.¹⁵ Mice were sensitized and challenged as previously described.^{16,17}

Administration of rosiglitazone, pioglitazone, GW9662, adenovirus vectors, SU5614, or SU1498

Rosiglitazone (5 mg/kg body weight/d; GlaxoSmithKline, Uxbridge, United Kingdom) dissolved in distilled water or pioglitazone (10 mg/kg body weight/d; Takeda Chemical Industries Ltd, Osaka, Japan) dissolved in dimethyl sulfoxide and diluted with 0.9% NaCl was administered 7 times by oral gavage at 24-hour intervals on days 19 to 25, beginning 2 days before the first challenge of ovalbumin. GW9662 (0.5 mg/kg body weight/d; Cayman, Ann

Arbor, Mich) dissolved in PBS was administered intratracheally 2 times to each animal, once on day 21 and the second time on day 25. Adenovirus vectors were administered intratracheally (10^9 plaque-forming units) 2 times to each animal, once on day 21 (1 hour before the first airway challenge with ovalbumin) and the second time on day 23 (3 hours after the last airway challenge with ovalbumin). Inhibitors of vascular endothelial growth factor (VEGF) receptor tyrosine kinase, SU5614 (Flk-1; IC_{50} = 1.2 μ mol/L, 5-chloro-3-[(3,5-dimethylpyrrol-2-yl)methylene]-2-indolinone; Calbiochem, San Diego, Calif), and SU1498 ([E]-3-[3,5-diisopropyl-4-hydroxyphenyl]-2-[(3-phenyl-n-propyl)amino-carbonyl]acrylonitrile; Calbiochem) were used to inhibit VEGF activity. SU5614 (2.5 mg/kg body weight/d) or SU1498 (9 mg/kg body weight/d) was dissolved in dimethyl sulfoxide, diluted with 0.9% NaCl solution, and administered intraperitoneally 3 times at 24-hour intervals, beginning 1 hour after the last airway challenge with ovalbumin.

Measurement of intracellular ROS

Reactive oxygen species were measured by a method previously described with modification.^{18,19} The numbers of ROS-positive cells stained by dichlorofluorescein and total cells were counted. Data of ROS levels were analyzed as the ratio of ROS-positive cells to total cells. We also performed FACScan analysis to measure ROS level with dichlorofluorescein-stained cells (1×10^4 cells) using a FACSCalibur (BD Biosciences, San Jose, Calif). The data were analyzed with the CellQuest Pro program (BD Biosciences) and expressed as ROS level per cell.

Measurement of T_H2 cytokines and VEGF

Levels of IL-4, IL-5, IL-13, and VEGF were quantified in the supernatants of bronchoalveolar lavage (BAL) fluids by enzyme immunoassays according to the manufacturer's protocol (IL-4 and IL-5; Endogen, Inc, Woburn, Mass; IL-13 and VEGF; R&D Systems, Inc, Minneapolis, Minn).

Western blot analysis

Protein expression levels were analyzed by Western blot analysis, as described previously.¹⁶

Measurement of plasma exudation

To assess lung permeability, Evans blue dye (EBD) was used as described previously.²⁰

RNA isolation and RT-PCR

Levels of mRNA expression were analyzed by RT-PCR assay using total RNA isolated from lung tissues by a rapid extraction method (TRI-Reagent; Sigma-Aldrich, St Louis, Mo) as previously described.²¹

Cytosolic or nuclear protein extractions for analysis of NF- κ B p65, PPAR γ , HIF-1 α , and HIF-1 β

Cytosolic or nuclear extraction was performed as described previously.²⁰

Densitometric analyses and statistics

All immunoreactive signals were analyzed by densitometric scanning (Gel Doc XR; Bio-Rad Laboratories Inc, Hercules, Calif). Data were expressed as means $\pm$ SEMs. Statistical comparisons were performed by using 1-way ANOVA followed by the Scheffé test. Significant differences between groups were determined by using the unpaired Student *t* test. Statistical significance was set at *P* < .05.

RESULTS

Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on ROS levels in BAL fluids

Numbers of ROS-positive cells in BAL fluids were increased significantly at 72 hours after ovalbumin inhalation compared with the numbers after saline inhalation (Fig 1, A). The increased numbers of ROS-positive cells were substantially reduced by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . In addition, FACSscan analysis showed that dichlorofluorescein intensity of cells in BAL fluids was increased at 72 hours after ovalbumin inhalation compared with the intensity after saline inhalation (Fig 1, B). The increased levels of dichlorofluorescein fluorescence intensity at 72 hours after ovalbumin inhalation were decreased after administration of PPAR γ agonists or AdPPAR γ . The inhibitory effect of rosiglitazone treatment on ROS generation in BAL fluids was abrogated when a PPAR γ antagonist, GW9662, was administered concomitantly with the agonist.

Effect of rosiglitazone, pioglitazone, or AdPPAR γ on PPAR γ protein levels in lung tissues of ovalbumin-sensitized and ovalbumin-challenged mice

Western blot analysis revealed that levels of PPAR γ in nuclear protein extracts of lung tissues were increased at 72 hours after ovalbumin inhalation compared with the levels in the control mice (Fig 2, A and B). The increased PPAR γ levels in nuclear protein extracts were further increased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . In contrast, the levels of PPAR γ protein in cytosol were decreased after ovalbumin inhalation compared with the levels in the control mice (Fig 2, A and B). The decreased PPAR γ levels in cytosol were further decreased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ .

Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on NF- κ B p65 protein levels in lung tissues of ovalbumin-sensitized and ovalbumin-challenged mice

Western blot analysis showed that levels of NF- κ B p65 in nuclear protein extracts of lung tissues were increased at 72 hours after ovalbumin inhalation compared with the levels in the control mice (Fig 2, C and D). The increased NF- κ B p65 levels in nuclear protein extracts were decreased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . In contrast, levels of NF- κ B p65 protein in cytosol from lung tissues were decreased after ovalbumin inhalation compared with the levels in the control mice (Fig 2, C and D). The decreased NF- κ B p65 levels in cytosol were increased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . No significant changes were observed in ovalbumin-sensitized and ovalbumin-challenged mice treated with GW9662 plus rosiglitazone or adenovirus vector expressing LacZ (AdLacZ) (Fig 2, C and D).

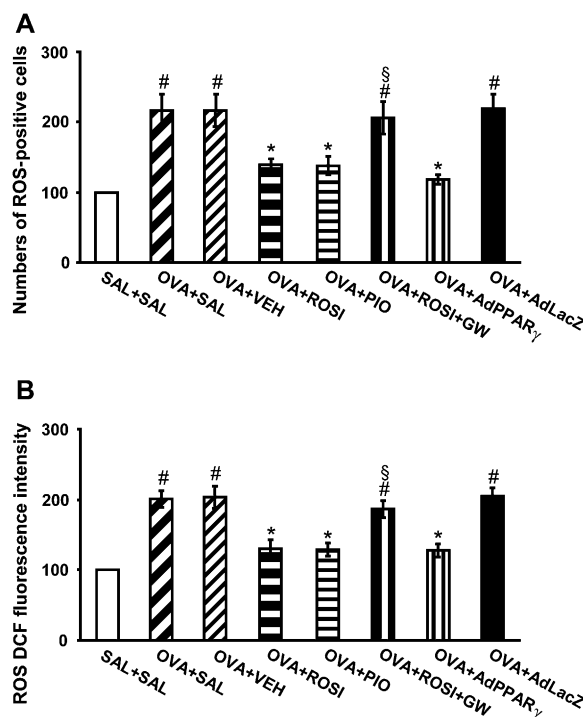


FIG 1. Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on ROS levels. **A**, Ratio of ROS-positive cells to total cells. **B**, dichlorofluorescein (DCF) fluorescence intensity. The ROS levels are presented as the relative ratio of values in ovalbumin (OVA)-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered GW9662 plus rosiglitazone (OVA+ROSI+GW), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), or OVA-inhaled mice administered AdLacZ (OVA+AdLacZ) to those in saline-inhaled mice administered saline (SAL+Sal). The ROS value in the BAL fluids of SAL+Sal is arbitrarily presented as 100. Bars represent means $\pm$ SEMs from 6 mice per group. [#] $P < .05$ versus SAL+Sal; ^{*} $P < .05$ versus OVA+Sal; [§] $P < .05$ versus OVA+ROSI.

Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on intercellular adhesion molecule 1, vascular cell adhesion molecule 1, eotaxin, and RANTES expression

Western blot analysis revealed that intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), eotaxin, and RANTES protein levels in lung tissues were increased significantly at 72 hours after ovalbumin inhalation compared with the levels after saline inhalation. The increased ICAM-1, VCAM-1, eotaxin, and RANTES levels were significantly reduced by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . However, no significant changes were observed in ovalbumin-sensitized and ovalbumin-challenged mice treated with GW9662 plus rosiglitazone or AdLacZ (see this article's Fig E1, A, B, E, and F, in the Online Repository at www.jacionline.org). In addition, RT-PCR analysis showed that levels of ICAM-1, VCAM-1, eotaxin, and

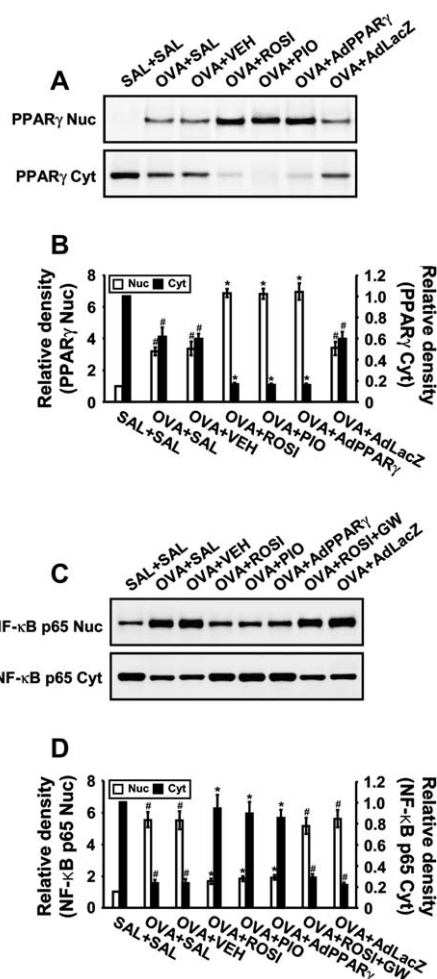


FIG 2. Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on levels of PPAR γ and NF- κ B p65. **A and C,** Western blotting of PPAR γ and NF- κ B p65 in nuclear (Nuc) and cytosolic (Cyt) protein extracts from lung tissues. **B and D,** Densitometric analyses are presented as the relative ratio of PPAR γ or NF- κ B p65 levels in ovalbumin (OVA)-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), OVA-inhaled mice administered GW9662 plus rosiglitazone (OVA+ROSI+GW), or OVA-inhaled mice administered AdLacZ (OVA+AdLacZ) to those in saline-inhaled mice administered saline (SAL+Sal). The PPAR γ or NF- κ B p65 level in nuclear protein extracts of SAL+Sal is arbitrarily presented as 1. Bars represent means $\pm$ SEMs from 6 mice per group. # P < .05 versus SAL+Sal; * P < .05 versus OVA+Sal.

RANTES mRNA were increased significantly after ovalbumin inhalation compared with the levels after saline inhalation. The increased levels of ICAM-1, VCAM-1, eotaxin, and RANTES mRNA were decreased significantly by the administration of rosiglitazone, pioglitazone, or AdPPAR γ (see this article's Fig E1, C, D, G, and H, in the Online Repository at www.jacionline.org).

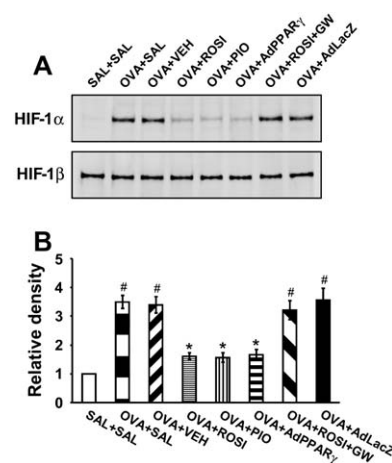


FIG 3. Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on HIF-1 α and HIF-1 β levels in nuclear protein extracts from lung tissues. **A,** Western blotting of HIF-1 α and HIF-1 β protein. **B,** Densitometric analyses are presented as the relative ratio of HIF-1 α to HIF-1 β . The relative ratio of HIF-1 α in nuclear protein extracts of saline-inhaled mice administered saline (SAL+Sal) is arbitrarily presented as 1. Bars represent means $\pm$ SEMs from 6 mice per group. # P < .05 versus SAL+Sal; * P < .05 versus ovalbumin (OVA)-inhaled mice administered saline (OVA+Sal). GW, GW9662; PIO, pioglitazone; ROSI, rosiglitazone; VEH, vehicle.

Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on HIF-1 α protein levels in lung tissues of ovalbumin-sensitized and ovalbumin-challenged mice

Western blot analysis revealed that levels of HIF-1 α in nuclear protein extracts from lung tissues were increased at 72 hours after ovalbumin inhalation compared with the levels in the control mice (Fig 3, A and B). The increased HIF-1 α levels in nuclear protein extracts were decreased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . In contrast, no significant changes were observed in ovalbumin-sensitized and ovalbumin-challenged mice treated with GW9662 plus rosiglitazone or AdLacZ.

Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, AdPPAR γ , SU5614, or SU1498 on VEGF expression of ovalbumin-sensitized and ovalbumin-challenged mice

Enzyme immunoassay showed that the levels of VEGF protein in BAL fluids were increased significantly at 72 hours after ovalbumin inhalation compared with the levels after saline inhalation and that administration of rosiglitazone, pioglitazone, AdPPAR γ , SU5614, or SU1498 dramatically reduced the increased levels of VEGF in BAL fluids (Fig 4, A). In contrast, no significant changes were observed in ovalbumin-sensitized and ovalbumin-challenged mice treated with GW9662 plus rosiglitazone or AdLacZ. Consistent with the results obtained from the enzyme immunoassay, Western blot analysis revealed that the administration of rosiglitazone, pioglitazone, or

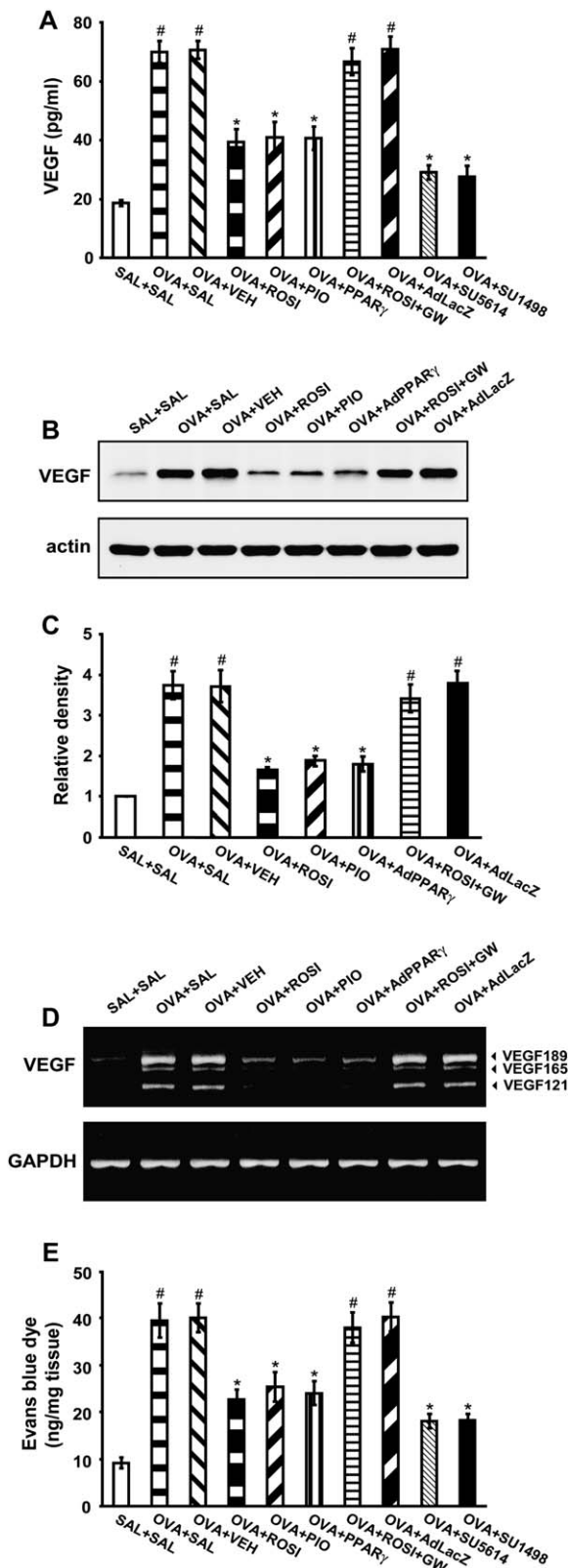


FIG 4. Effects of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, AdPPAR γ , SU5614, or SU1498 on VEGF expression and on plasma exudation. Sampling was performed in saline-inhaled mice administered saline (SAL+Sal), ovalbumin (OVA)-inhaled mice

AdPPAR γ reduced significantly the increased levels of VEGF in the lung tissues at 72 hours after ovalbumin inhalation (Fig 4, B and C). In addition, RT-PCR analysis revealed that VEGF mRNA expression in lung tissues was increased after ovalbumin inhalation compared with the level after saline inhalation. The increased VEGF mRNA expression was decreased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ (Fig 4, D).

Rosiglitazone, pioglitazone, AdPPAR γ , SU5614, or SU1498 reduced plasma extravasation in ovalbumin-sensitized and ovalbumin-challenged mice

EBD assay revealed that plasma extravasation was significantly increased at 72 hours after the last challenge. The increase in plasma extravasation was significantly reduced by the administration of rosiglitazone, pioglitazone, AdPPAR γ , SU5614, or SU1498 (Fig 4, E).

DISCUSSION

Bronchial asthma is an inflammatory disease of the airways characterized by airway obstruction and increased airway responsiveness.¹ Oxidative stress plays a critical role in the pathogenesis of airway inflammation.² PPAR γ agonists, which are known to modulate oxidative stress, are involved in the regulation of inflammatory responses.^{12-14,22} Our current study with ovalbumin-induced murine model of allergic airway disease has revealed that ROS production in cells from BAL fluids was increased, and that administration of the PPAR γ agonists, rosiglitazone or pioglitazone, or AdPPAR γ reduced the increased ROS levels; the increased expression of adhesion molecules, chemokines, T_H2 cytokines, and VEGF; airway inflammation; and bronchial hyperresponsiveness. Our results also indicate that PPAR γ agonists or AdPPAR γ downregulate NF- κ B activity and that they also reduce VEGF expression as well as HIF-1 α activation. Therefore, we suggest that PPAR γ agonists or AdPPAR γ modulate ROS generation and inhibit NF- κ B signal transduction pathway and HIF-1 α activity that enhance VEGF expression in allergic airway disease of mice.

administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROS), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), OVA-inhaled mice administered GW9662 plus rosiglitazone (OVA+ROS+GW), OVA-inhaled mice administered AdLacZ (OVA+AdLacZ), OVA-inhaled mice administered SU5614 (OVA+SU5614), and OVA-inhaled mice administered SU1498 (OVA+SU1498). **A**, Enzyme immunoassay of VEGF. **B**, Western blotting of VEGF protein. **C**, Densitometric analyses are presented as the relative ratio of VEGF to actin. The relative ratio of VEGF in the lung tissues of SAL+Sal is arbitrarily presented as 1. **D**, RT-PCR of VEGF mRNA. **E**, Measurement of plasma exudation using EBD. Bars represent means $\pm$ SEMs from 6 mice per group. [#]*P* < .05 versus SAL+Sal; ^{*}*P* < .05 versus OVA+Sal. GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

Recent studies suggest the use of PPAR γ ligands for the possible treatment of bronchial asthma.^{11,17,23} Upregulation of PPAR γ expression is observed in human asthmatic airways.^{9,17} In animal models of allergic airway disease, activation of PPAR γ induces an inhibition of eosinophil and lymphocyte influx^{17,23} and decreases antigen-induced airway hyperresponsiveness, lung inflammation, eosinophilia, and GATA-3 expression.¹¹ Furthermore, PPAR γ is expressed in eosinophils, and its activation inhibits *in vitro* chemotaxis and antibody-dependent cellular cytotoxicity.¹¹ Consistent with these observations, our results have shown that administration of the PPAR γ agonists or AdPPAR γ substantially inhibited expression of T_H2 cytokines, adhesion molecules, and chemokines; bronchial hyperresponsiveness; and airway inflammation. Therefore, we conclude that regulation of PPAR γ may play an important role in the induction and maintenance of allergic airway disease in mice.

Oxidative stress is caused by a large variety of free oxygen radicals known as ROS. ROSs released by eosinophils and other leukocytes infiltrating the airways play an important role in the airway tissue injury observed in asthma.² An excess production of ROS and defective endogenous anti-oxidant defense mechanisms may be present in asthma. Evidence for increase of oxidative stress in asthma is further provided by the finding of defective endogenous antioxidant capacity in patients with asthma.² Previous reports have shown that the production of ROS is increased in the airways of ovalbumin-challenged animals.^{24,25} Although the lung has a well developed antioxidant system,²⁶ overproduction of ROS or depression of the protective system results in epithelial cell damage, cell shedding, and bronchial hyperreactivity.²⁷ Studies with animal models have indicated that ROSs contribute to airway hyperresponsiveness by increasing vagal tone caused by damage of oxidant sensitive β -adrenergic receptors as well as decreasing mucociliary clearance.^{28,29} These findings suggest that ROSs are involved in the regulation of airway inflammation and bronchial hyperresponsiveness. Several studies have shown that PPAR γ agonists reduce the lesions associated with ischemia-reperfusion of the stomach, kidney, heart, and gut, possibly because of the anti-inflammatory property.¹²⁻¹⁴ Other investigators have also demonstrated that PPAR γ agonist reduces the oxidative stress production via the modulation in the activities of oxidative stress-related enzymes as well as the anti-inflammatory mechanisms in several ischemia/reperfusion models.^{22,30} Consistent with these observations, we have shown that the increased ROS levels in BAL cells of an ovalbumin-induced airway inflammatory model were substantially reduced by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . In this study, data of ROS levels were analyzed as the ratio of ROS-positive cells to total cells. In addition, dichlorofluorescein fluorescence intensity was also decreased after administration of PPAR γ agonists or AdPPAR γ . Thus, these results show that the reduction in ROS levels is not simply a result of reduction in the influx of inflammatory cells that produce the ROS. These observations are supported by recent

reports that PPAR γ agonists diminished ROS generation directly.^{31,32} Taken together, we suggest that ROS is reduced directly and/or indirectly through inhibition of inflammation by activation of PPAR γ .

A recent study has demonstrated that the pioglitazone inhibits intracellular superoxide radical generation and activation of the redox-sensitive transcription factor.³¹ NF- κ B plays a critical role in immune and inflammatory responses, including asthma.³³⁻³⁵ Development of oxidant/antioxidant imbalance in asthma leads to activation of NF- κ B.³⁵ Consistent with these observations, NF- κ B protein levels in nuclear protein extracts from lung tissues were substantially increased and the increased levels of NF- κ B were decreased by administration of antioxidant drugs, L-2-oxothiazolidine-4-carboxylic acid, or α -lipoic acid^{3,36} in the ovalbumin-induced model of allergic airway disease used for the current study (see this article's Figs E2 and E3 in the Online Repository at www.jacionline.org). Also, it is known that activation of this transcription factor induces a variety of inflammatory genes that are abnormally expressed in asthma.^{35,37,38} In this study, we have found that expression of adhesion molecules, chemokines, and T_H2 cytokines was increased significantly after allergen challenge and the increased expression of these molecules was decreased dramatically by administration of an inhibitor of NF- κ B activation, BAY 11-7085, in a murine model of allergic airway disease (see this article's Fig E4 and E5 in the Online Repository at www.jacionline.org). Administration of PPAR γ agonists or AdPPAR γ resulted in significant reduction of NF- κ B translocation into nucleus and of expression of these adhesion molecules, chemokines, and cytokines. These results indicate that PPAR γ agonists or AdPPAR γ inhibit NF- κ B activity by preventing translocation of this transcription factor into a nucleus that is induced by ROS. ROSs have been directly implicated as second messengers in the activation of NF- κ B, based on the ability of oxidants to activate NF- κ B via oxidation of its cysteine sulfhydryl group or by ubiquitination and proteolysis.³⁹ Inhibitory κ B has 5 to 7 conserved domains known as ankyrin repeats, each consisting of around 30 amino acids, forming a unit that is able to interact with NF- κ B subunits, thus masking the nuclear localization signals and preventing activation of NF- κ B and its translocation to the nucleus.³³ Antioxidants have been known to inhibit NF- κ B activation by preventing inhibitory κ B degradation in response to various stimuli.⁴⁰⁻⁴² Therefore, we suggest that PPAR γ agonists or AdPPAR γ inhibit NF- κ B signal transduction pathway by decreasing NF- κ B binding activity to the promoter regions of many genes which may induce airway inflammation in allergic airway disease.

Hypoxia-inducible factor 1 is a transcriptional activator that mediates changes in gene expression in response to changes in cellular oxygen concentrations.⁴³ Activation of hypoxia-responsive genes including VEGF is mediated by HIF-1, a heterodimeric basic helix-loop-helix-Per-Arnt/AhR-Sim domain transcription factor.⁴⁴ HIF-1 is composed of 2 subunits, HIF-1 α and HIF-1 β . While the β -subunit protein is present, the stability of the α -subunit

and its transcriptional activity are controlled by the intracellular oxygen concentration.^{45,46} Previous reports have demonstrated that HIF-1 α plays a critical role in immune and inflammatory responses,⁴⁷ and that ROSs stabilize HIF-1 α during hypoxia and/or nonhypoxia.^{48,49} Consistent with these observations, determination of HIF-1 α protein levels in nuclear extracts has revealed that these protein levels were substantially increased after ovalbumin inhalation, and the increased levels of HIF-1 α protein were decreased by the administration of antioxidants, L-2-oxothiazolidine-4-carboxylic acid, or α -lipoic acid in an ovalbumin-induced model of allergic airway disease (see this article's Fig E6 in the Online Repository at www.jacionline.org). The administration of PPAR γ agonists or AdPPAR γ resulted in a significant reduction of nuclear HIF-1 α level as well as expression of VEGF. In addition, the increased expression of VEGF after ovalbumin inhalation was decreased by the administration of a potent inhibitor for HIF-1 α , 2-methoxyestradiol (see this article's Fig E7 and Fig E8 in the Online Repository at www.jacionline.org). These findings are supported by the previous report that VEGF expression is regulated through HIF-1 α expression,⁴⁴ and these results suggest that PPAR γ agonists and AdPPAR γ inhibit VEGF expression in allergic airway disease that leads to an increase in HIF-1 α activity and that they downregulate HIF-1 α activation by reducing ROS generation. Exudation of plasma proteins into the airways plays a role in airway inflammation and hyperresponsiveness. VEGF has been implicated to contribute to asthmatic tissue edema through its effect on vascular permeability.^{50,51} VEGF is one of the crucial mediators that can induce altered vascular permeability.^{20,52} In the current study, we have found that VEGF expression and plasma exudation were increased after ovalbumin inhalation, and the increased levels of VEGF and plasma exudation were decreased significantly after administration of VEGF receptor inhibitors, which reduce VEGF activity in ovalbumin-induced allergic airway disease of mice.

Reports showing an increase of oxidative stress in asthma and indicating a potential role of PPAR γ in pathogenesis of the diseases have accumulated over the years, and it is now well established that VEGF plays a critical role in asthma.^{53,54} On the basis of these observations, we have administered PPAR γ agonists or AdPPAR γ to allergen-challenged mice. Administration of PPAR γ agonists or AdPPAR γ resulted in a significant reduction of all pathophysiological symptoms examined. Our data suggest that PPAR γ modulates intracellular ROS generation, VEGF expression through regulation of HIF-1 α activity, and activation of the redox-sensitive transcription factor NF- κ B (see this article's Fig E9 in the Online Repository at www.jacionline.org). Thus, these findings provide a rationale for the use of PPAR γ agonists to prevent and or treat asthma and other airway inflammatory disorders.

We thank Professor Mie-Jae Im for critical reading of the manuscript.

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