

DR. JI HYEON RYU (Orcid ID : 0000-0003-0342-3108)

PROF. YONG-IL SHIN (Orcid ID : 0000-0001-7894-0930)

Article type : Letter to the Editor

Light-emitting diode-based photobiomodulation reduces features of allergic asthma in mice

Short title: Photobiomodulation reduces allergic asthma in mice

Keywords: airway smooth muscle cell, allergic asthma, LED therapy, photobiomodulation

To the Editor,

Approximately 300 million people worldwide suffer from asthma: this prevalence is rapidly increasing,¹ imposing a huge economic burden on healthcare costs. Currently, there is no cure for allergic asthma; thus, therapies are only effective in treating symptoms by controlling inflammatory processes without altering the underlying immune activation.² Inhaled corticosteroids (ICSs) are currently the mainstay of asthma therapy.³ Although anti-inflammatory and immunosuppressive effects of ICSs can prevent the life-threatening consequences of asthma, their long-term therapeutic effects are limited with respect to airway remodeling,⁴ and some individuals develop resistance to the therapeutic effects of corticosteroids. Therefore, it is imperative to find new safer, less toxic, and more effective therapies for asthma.

Light-emitting diode (LED)-based photobiomodulation (L-PBM) is a non-painful, inexpensive, easy-to-use, and safe therapeutic intervention⁵ with no side effects even after prolonged use. L-PBM shows great potential as a new treatment modality for several diseases including pulmonary diseases.⁶ Cytochrome c oxidase (Cco) is a primary photoreceptor of photosignals and catalyzes nitric oxide (NO) synthesis.⁷ In asthma, NO has

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13929

This article is protected by copyright. All rights reserved.

both beneficial and detrimental effects. NO can act on postcapillary venule leading to plasma extravasation and increase allergic inflammatory responses, and high levels of NO in asthmatic patients are observed.⁸ Nevertheless, NO produced by constitutive enzyme has beneficial effects, causing bronchodilation by relaxing airway smooth muscle via regulation of non-adrenergic non-cholinergic nerves and cyclic guanosine monophosphate.^{8,9}

The activity of Cco/NO peaks at a wavelength range of orange color,⁷ whose wavelength interval range is 590–630 nm. Here, we hypothesized that wavelength of 610 nm, the center value of orange color, would induce airway smooth muscle relaxation with increased NO production in asthma. We evaluated the effects of L-PBM using a 610 nm LED in an ovalbumin (OVA)-induced mouse model of allergic asthma and compared them with those of dexamethasone (DEX), a corticosteroid typically used to treat asthma.

Female BALB/c mice were sensitized and challenged with OVA for 5 weeks (Figure 1A). An LED was used for PBM at 1 and 4 h after each challenge with OVA (Figure S1). L-PBM was applied to the respiratory tract via direct contact with the skin for 20 min. The materials and methods used in this study are detailed in the Supporting Information.

In this study, sham-L-PBM does not affect asthmatic parameters (Figure S2). To examine the effects of L-PBM on airway inflammation induced by OVA exposure, total and differential cells in the bronchoalveolar lavage fluid (BALF) of mice were determined. The increase in the numbers of total and differential cells in BALF of OVA mice was reduced by DEX or L-PBM (Figure 1B, C). Inflammatory cell infiltration in the peribronchial areas of the lung was observed in OVA mice, which was attenuated by DEX or L-PBM (Figure 1D). The OVA-challenged mice showed a significant increase in AHR on exposure to methacholine, which was significantly reduced by DEX or L-PBM at 25 and 50 mg/mL of methacholine (Figure 1E). The OVA challenge increased the levels of total and OVA-specific immunoglobulin E (IgE) in the serum, which was ameliorated by L-PBM (Figure 1F). L-PBM decreased Th2 cytokines [interleukin (IL)-4, IL-5, IL-13] (Figure 1G), and increased interferon- γ levels in BALF (Figure 1H). The levels of bronchoconstrictive mediators, such as histamine, tryptase, and leukotriene E4 in BALF increased in the OVA group and were profoundly attenuated by L-PBM or DEX (Figure 1I-K). Of note, the inhibitory effects of L-PBM on airway inflammation, AHR, serum IgE levels, Th2 cytokine concentrations, and bronchoconstrictive mediators in the BALF were similar to those of DEX.

Persistent airway inflammation leads to structural changes known as airway remodeling. Therefore, we assessed whether L-PBM could improve features of airway remodeling. Goblet cell overproduction was observed in the bronchial epithelium of OVA mice (Figure 2A), which was reduced by L-PBM, with similar effects to that of DEX treatment. Increased deposition of collagen fibers was noted around the lung's peribronchial areas in OVA mice (Figure 2B). However, L-PBM reduced OVA-induced collagen deposition. Airway smooth muscle thickness was increased in OVA mice, which was decreased by the L-PBM (Figure 2C). Noteworthy, contrary to L-PBM, DEX did not change the collagen deposition and airway smooth muscle thickness increased by OVA.

To examine the effect of L-PBM on the contraction of airway smooth muscle cells (ASMCs), changes in Ca^{2+} levels and collagen gel contractions were examined in ASMCs isolated from each experimental group. Basal intracellular Ca^{2+} levels in ASMCs isolated from the OVA group were higher than those from PBS+L-PBM group (Figure 2D). Histamine induced an increase in the Ca^{2+} levels; however, ASMCs isolated from the OVA+L-PBM group showed significant attenuation in the histamine-induced increase in Ca^{2+} levels compared to the OVA or OVA+DEX groups (Figure 2E). To identify the effect of L-PBM on the histamine-induced contraction of ASMCs, a gel contraction assay was performed. Histamine added to ASMC gels in the OVA group increased contractility by 4.6-fold compared to that in the PBS+L-PBM group (Figure 2F). However, the ASMC gels in the OVA+L-PBM group showed reduced histamine-induced contraction. However, DEX did not affect histamine-induced ASMC contraction.

The thymus and spleen modulate the immune system, and their weights indicate their functional properties.¹⁰ To identify the effect of L-PBM on the weight of immune organs in OVA-induced asthmatic mice, thymus and spleen weights were measured. Significant atrophy of the thymus and spleen was observed in the DEX-treated mice (Figure S1A, C), but histopathological changes such as alterations in the ratio of cortex to medulla in the thymus and the ratio of red pulp to white pulp in the spleen were not observed in all experimental groups (Figure S1B). However, L-PBM did not affect the size of these organs compared to the control and OVA groups. Therefore, L-PBM demonstrated a similar or superior suppression effect than DEX on the asthmatic phenotype by modulating specific immune activities without inducing immunosuppression.

We examined mitogen-activated protein kinase (MAPK) and nuclear factor- κ B (NF- κ B) activation in lung tissues obtained from asthmatic mice. L-PBM inhibited p-ERK, p-p38, and p-JNK levels and NF- κ B p65 protein translocation into the nucleus compared to that in the OVA group (Figure S2).

Overall, our results demonstrated that L-PBM could effectively suppress asthmatic parameters in a mouse model of allergic asthma. These beneficial effects of L-PBM may be mediated by inhibition of Th2 response and bronchoconstrictive mediators via suppression of the MAPK/NF- κ B signaling pathway. Additionally, endogenous NO produced by L-PBM seems to affect airway smooth muscle relaxation; however, further detailed studies are needed to uncover the underlying cellular and molecular mechanisms. L-PBM could hence be a potential novel therapeutic approach and an effective alternative to corticosteroids in asthma treatment.

Ji Hyeon Ryu¹, Yeonye Kim¹, Taehyung Kim¹, Young Mi Kim¹, Jaehoon Jung¹, Sang-Yull Lee², Seung Eun Lee³, Nam Gyun Kim^{4,*}, Yong-Il Shin^{1,5,*}

¹Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan 50612, Korea

²Department of Biochemistry, Pusan National University School of Medicine, Yangsan 50612, Korea

³Department of Respiratory and Allergy, Pusan National University Yangsan Hospital, Yangsan 50612, Korea

⁴Medical Research Center of Color Seven, Seoul 06719, Korea

⁵Department of Rehabilitation Medicine, Pusan National University School of Medicine, Yangsan 50612, Korea

* These authors contributed equally to this work.

Correspondence:

Yong-Il Shin, MD, PhD

Department of Rehabilitation Medicine, Pusan National University School of Medicine, Yangsan 50612, Korea.

Tel.: +82-55-360-2872; Fax: +82-55-360-4251; E-mail: rmshin@pusan.ac.kr

Nam Gyun Kim, PhD

Medical Research Center of Color Seven. Seoul 06719, Korea. Tel.: +82-1577-5691; E-mail: nespris@naver.com

Acknowledgements

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science & ICT(NRF-2017M3A9E9032772). This research was also supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI17C2397).

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

YIS and NGK conceived and designed the experiments. JHR, YK, and YMK performed experiments and analyzed the data. TK, JJ, SEL, and SYL analyzed and interpreted the data. JHR and YIS wrote the paper. YIS and NGK supervised the study and reviewed the manuscript.

References

1. Network GA. The global asthma report 2014. *Auckland, New Zealand*. 2014;769.
2. de Lima FM, Villaverde A, Albertini R, et al. Dual Effect of low-level laser therapy (LLLT) on the acute lung inflammation induced by intestinal ischemia and reperfusion: Action on anti- and pro-inflammatory cytokines. *Lasers Surg Med*. 2011;43:410-420.
3. Suissa S, Ernst P. Inhaled corticosteroids: impact on asthma morbidity and mortality. *J Allergy Clin Immunol*. 2001;107:937-944.
4. Caramori G, Groneberg D, Ito K, et al. New drugs targeting Th2 lymphocytes in asthma. *J Occup Med Toxicol*. 2008;3:S6.
5. Yeh NG, Wu C-H, Cheng TC. Light-emitting diodes—their potential in biomedical applications. *RENEW SUST ENERG REV*. 2010;14:2161-2166.

6. Siqueira VPC, Evangelista MIS, dos Santos A, et al. Light-Emitting Diode treatment ameliorates allergic lung inflammation in experimental model of asthma induced by ovalbumin. *Journal of biophotonics*. 2017;10:1683-1693.
7. Ball KA, Castello PR, Poyton RO. Low intensity light stimulates nitrite-dependent nitric oxide synthesis but not oxygen consumption by cytochrome c oxidase: Implications for phototherapy. *Journal of Photochemistry and Photobiology B: Biology*. 2011;102:182-191.
8. Prado CM, Martins MA, Tibério IF. Nitric oxide in asthma physiopathology. *ISRN allergy*. 2011;2011.
9. Barnes P. NO or no NO in asthma? *Thorax*. 1996;51:218-220.
10. Basketter D, Bremmer J, Buckley P, et al. Pathology considerations for, and subsequent risk assessment of, chemicals identified as immunosuppressive in routine toxicology. *Food and chemical toxicology*. 1995;33:239-243.

Figure legends

Figure 1. Inhibitory effect of L-PBM on ovalbumin (OVA)-induced airway inflammation.

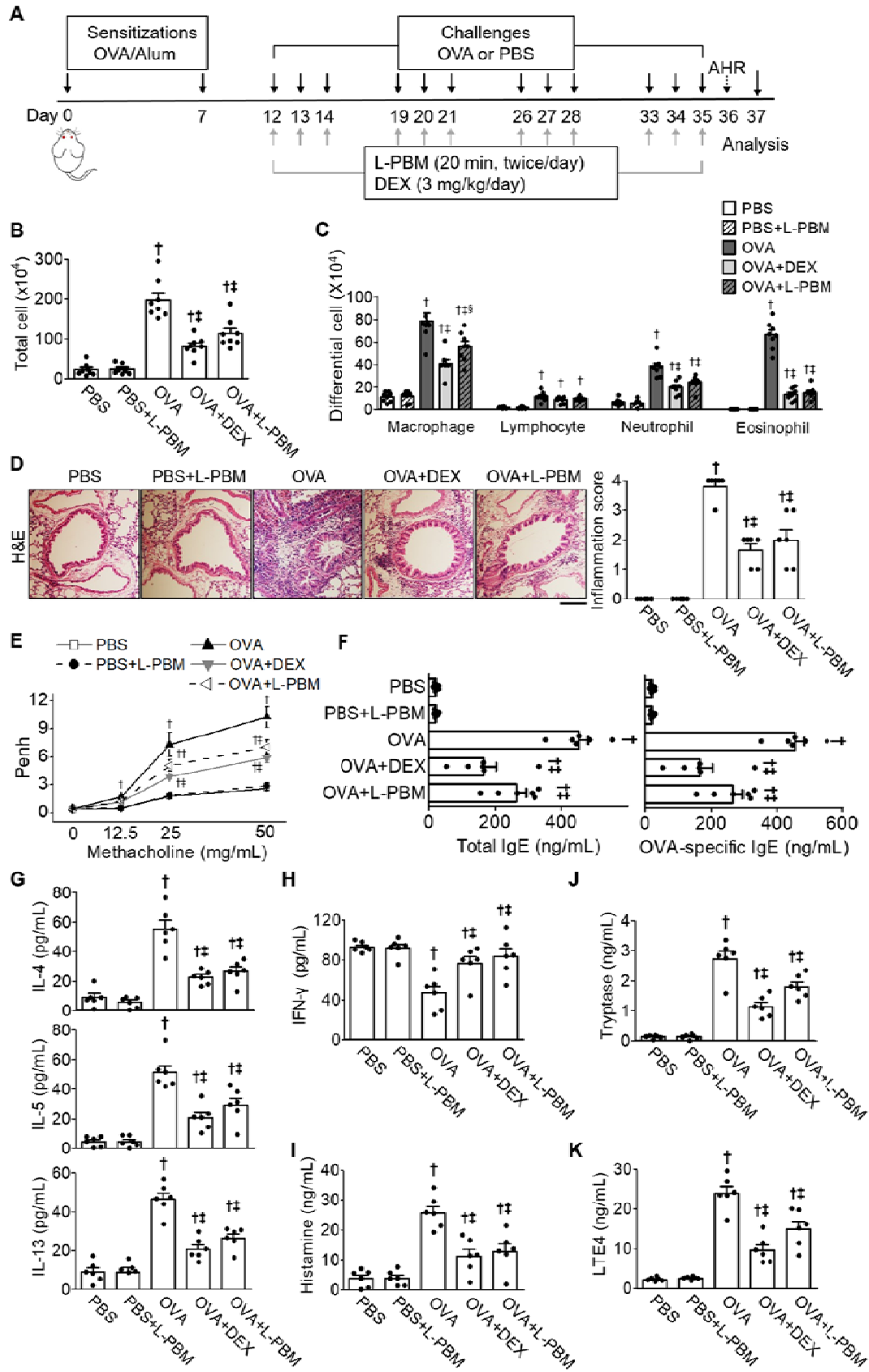
(A) Experimental scheme. (B) Total and (C) differential cell counts in BALF. (D) Representative images of H&E staining of lung tissues. Scale bar=100 μ m. (E) AHR to methacholine challenge. (F) Total and OVA-specific IgE in serum. (G) IL-4, IL-5, and IL-13, (H) IFN- γ , (I) histamine, (J) tryptase, and (K) LTE4 levels in BALF (n=10). [†] $p < 0.05$, compared to PBS + L-PBM. [‡] $p < 0.05$, compared to OVA. [§] $p < 0.05$, compared to OVA + DEX.

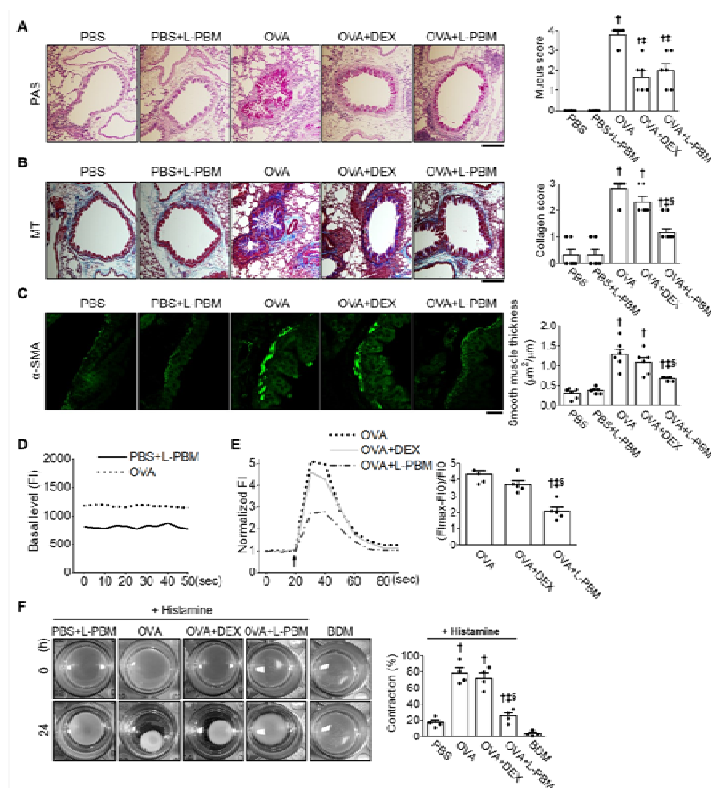
Abbreviations: L-PBM, light-emitting diodes-based photobiomodulation; BALF, bronchoalveolar lavage fluid; H&E, hematoxylin and eosin; AHR, airway hyperresponsiveness; IgE, immunoglobulin E; IL, interleukin; IFN- γ , interferon- γ ; LTE4, leukotriene E4; DEX, dexamethasone

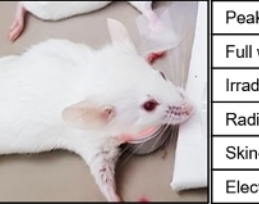
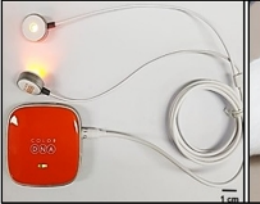
Figure 2. Reduction of OVA-induced airway remodeling by L-PBM.

(A) Representative images of PAS and (B) MT staining of the lung tissues. Scale bar=100 μ m. (C) Immunostaining of lung sections with α -SMA antibody (n=8). Scale bar=20 μ m. (D) Basal Ca²⁺ levels of ASMCs in PBS + L-PBM or OVA groups. (E) Histamine-induced Ca²⁺ response in ASMCs isolated from different groups (n=10). (F) Representative images of collagen matrices in a collagen gel contraction assay (n=4). [†] $p < 0.05$, compared to PBS + L-PBM. [‡] $p < 0.05$, compared to OVA. [§] $p < 0.05$, compared to OVA + DEX.

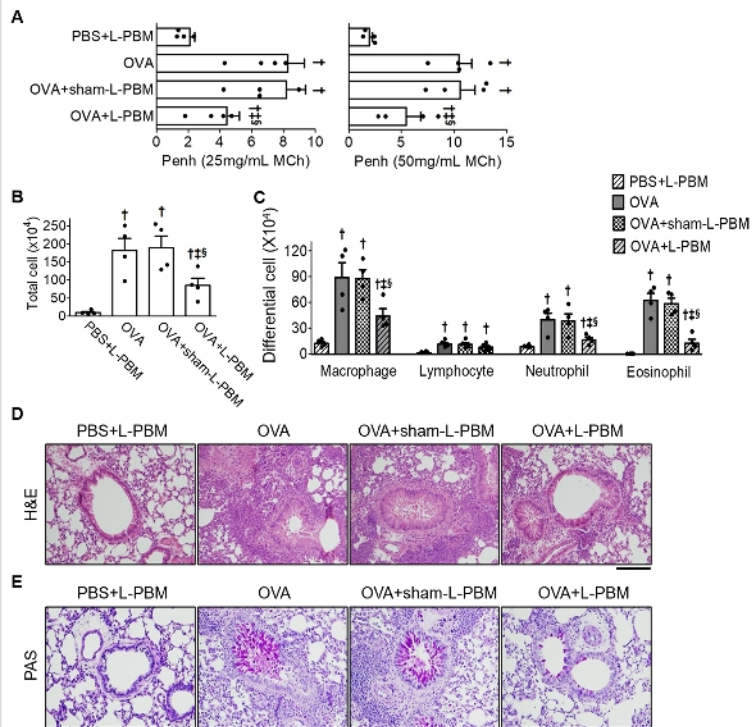
Abbreviations: PAS, periodic acid–Schiff; MT, Masson’s trichrome; α -SMA, alpha-smooth muscle actin; ASMCs, airway smooth muscle cells.

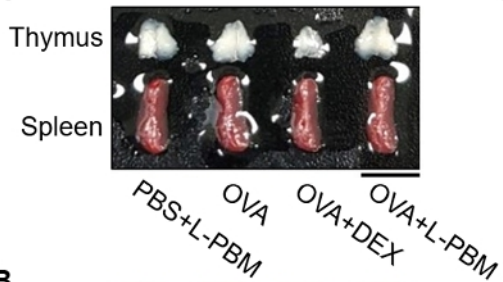
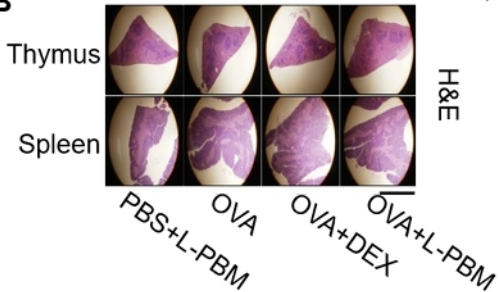
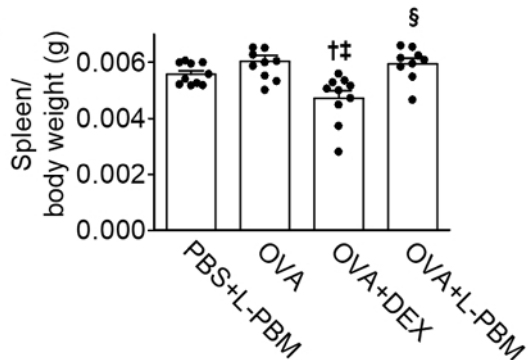
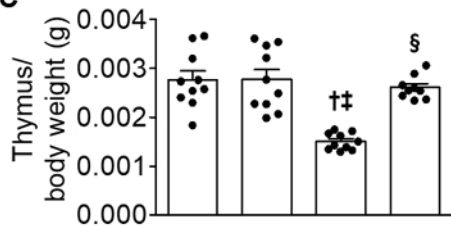


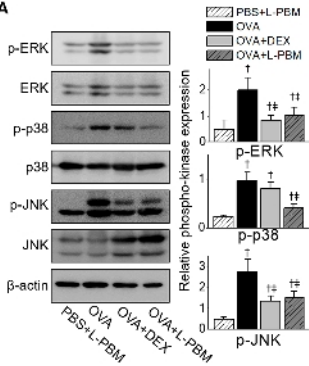
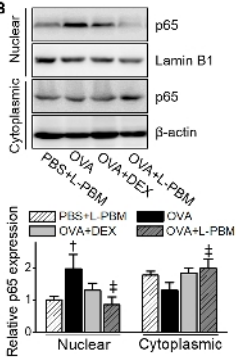




Peak wavelength	610 nm
Full width half maximum	24 nm
Irradiance	1.7 mW/cm ²
Radiant exposure	2.0 J/cm ²
Skin-adhesive light-emitting	
Electrodes: surface area, 1.6 cm; spot size, 0.4 cm diameter	



A**B****C**

A**B**

SUPPORTING INFORMATION

Materials and Methods

Mouse models of allergic asthma

Female BALB/c mice (six weeks of age) were purchased from Koatech Co. (Pyongtaec, Korea). The mice were housed in air-conventional animal housing and given food and water *ad libitum*. All experiments were approved by the Institutional Animal Care and Use Committee of Pusan National University in accordance with the National Institutes of Health Guidelines (PNU-2017-1668). The mice were randomly divided into the following five experimental groups (n = 8–10): phosphate-buffered saline (PBS) group comprising mice challenged with PBS, a PBS + L-PBM group comprising mice challenged with PBS and irradiated with L-PBM, an OVA group comprising mice challenged with OVA, an OVA + DEX group comprising mice challenged with OVA and treated with DEX (3 mg/kg/day), and an OVA + L-PBM group comprising mice challenged with OVA and irradiated with L-PBM. All mice were sensitized with an intraperitoneal injection of a mixture containing 100 µg OVA [5 mg/mL prepared in PBS; EndoGrade ovalbumin, Hyglos GmbH, Regensburg, Germany] and 2 mg aluminum hydroxide (alum; Invivo Gen, San Diego, CA, USA) in 0.2 mL PBS on days 0 and 7. Mice were then anesthetized with isoflurane (2% induction and 1.5% maintenance in 80% N₂O and 20% O₂) and received an intranasal challenge with 50 µg OVA in 30 µL PBS thrice a week for four weeks. DEX (25 mg/mL in distilled water; Sigma, St. Louis, Mo. USA) was intraperitoneally injected 1 h prior to the OVA challenge. Twenty-four hours after the last challenge, lung function was measured, and all mice were sacrificed at 48 h after the last intranasal challenge.

Light-emitting diode-based photobiomodulation (L-PBM)

An LED-based device (width × length × height; 4.9 × 4.9 × 1.3 cm, Color Seven Co., Seoul, Korea) was used for L-PBM with the following parameters: wavelength, 610 nm [full width at half maximum, 24 nm (orange color)]; irradiance, 1.7 mW/cm²; radiant exposure, 2.0 J/cm²; surface area of electrodes, 1.6 cm; and spot size of electrodes, 0.4 cm diameter (Figure S1). Light stimulation was applied to the respiratory tract via direct contact with the skin twice a day for 20 min at 1 and 4 h after each challenge with OVA or PBS concurrently under isoflurane anesthesia. The PBS groups were anesthetized for 20 min without L-PBM.

Measurement of airway hyperresponsiveness (AHR)

AHR to inhaled methacholine was assessed 24 h after the last challenge using whole-body plethysmography (OCP 3000, Allmedicus, Gyeonggi, Korea). Mice were exposed to various concentrations of aerosolized methacholine (Sigma; 0, 12.5, 25, and 50 mg/mL in PBS) using a nebulizer (HARVARD73-1963, Harvard Apparatus, MA, USA) for 3 min. The mice were returned in chambers, and measurements were obtained for 150 s after the completion of each nebulization.

Total and differential cell counts in bronchoalveolar lavage fluid (BALF)

BALF was collected as described previously.¹ Mice tracheas were intubated with an indwelling needle to wash the lungs with 1 mL of PBS. Total inflammatory cell numbers were assessed by counting cells in a hemocytometer after excluding dead cells by trypan blue staining, and the BALF was centrifuged at 200 *g* for 10 min at 4°C. The supernatants were stored at -70°C for subsequent cytokine assays. For differential cell counts, the cells in the BALF were centrifuged onto slides using cytocentrifugation (Cytospin, Thermo Shandon, Pittsburgh, PA, USA) and stained with Diff-Quick (Sysmex International Reagents, Kobe, Japan)

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of serum IgE were determined using the mouse IgE ELISA kit (Bethyl Laboratories, Montgomery, TX, USA) and the OVA-specific IgE ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer's instructions. The levels of cytokines (IL-4, IL-5, IL-13, and interferon- γ (IFN- γ); BD Biosciences, Franklin Lakes, NJ, USA), histamine (Enzo Life Sciences, Ann Arbor, MI, USA), tryptase (Cusabio Biotech., Wuhan, Hubei, China), and leukotriene E4 (LTE4; MyBioSource, San Diego, CA, USA) in the BALF were also quantified using respective ELISA kits per the manufacturer's instructions. The optical densities of the total and OVA-specific IgE, cytokines, histamine, tryptase, and LTE4 at 450 nm were measured on a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Histopathological examination

The lungs were fixed in 4% paraformaldehyde in 0.1 M PBS, embedded in paraffin and sectioned (5 μ m thick). Lung inflammation was evaluated using hematoxylin and eosin (H&E) staining. Mucus production was analyzed using the periodic acid-Schiff (PAS) staining kit (Millipore, Billerica, MA, USA). Collagen deposition in the lung was analyzed using the Masson's trichrome (MT) staining kit (Polysciences, Warrington, PA, USA). The stained sections were photographed using a BX-50 microscope (Olympus Co., Tokyo, Japan).

Lung inflammation was graded based on the following five-point scoring system: 0, no cells; 1, few cells; 2, ring of cells 1 cell layer deep; 3, ring of cells 2–4 cell layers deep; and 4, ring of cells >4 cell layers deep.² Mucin-positive goblet cells was quantified using the following five-point scoring system: 0, <5% PAS-positive cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; and 4, >75%.³ Collagen deposition in the peribronchiolar region was quantified using the following four-point scoring system: 0, no collagen deposition; 1, a thin layer of collagen; 2, a cluster of collagen; and 3, a thick layer of collagen.⁴

Immunohistochemistry

To determine the thickness of the airway smooth muscle mass, after deparaffinization, lung sections were incubated overnight at 4°C with monoclonal anti-actin and anti-smooth muscle actin (α -SMA)-FITC antibody (1:500; Sigma). The α -

SMA immunostaining area was observed using confocal laser scanning microscopy (Olympus, Tokyo, Japan) and quantified to evaluate airway smooth muscle layer thickness.⁵ Five sections were randomly selected, and quantifications were performed using ImageJ software (National Institute of Health, Bethesda, MD, USA).

Western blotting

Proteins isolated from mouse lung tissues were prepared into total protein, nuclear fractions, and cytoplasmic fractions. The lung tissues were homogenized in RIPA lysis buffer (GenDEPOT, Barker, TX, USA) containing protease inhibitors to obtain total protein. Nuclear and cytosolic proteins were prepared using the nuclear and cytoplasmic protein extraction kit (Bio-world, Dublin, Ohio, USA) according to the manufacturer's instructions. The membranes were blocked with 5% fat-free dry milk and incubated overnight at 4°C with the following specific primary antibodies: p-ERK (1:2000; Cell Signaling, Danvers, MA, USA), ERK (1:1000; Cell Signaling), p-p38 (1:1000; Cell Signaling), p38 (1:1000; Cell Signaling), p-JNK (1:1000; Cell Signaling), JNK (1:1000; Cell Signaling), and NF-κB (1:1000; Cell Signaling). The membranes were then washed and incubated with secondary antibody conjugated with horseradish peroxidase. Immuno-positive bands were visualized using the Western Lightning® Plus-enhanced chemiluminescence kit (PerkinElmer, LAS Inc., Waltham, MA, USA) and a LAS-3000 luminescence image analyzer (Fujifilm, Tokyo, Japan). Band intensity was quantified using ImageJ software (NIH). The membranes were stripped and incubated with β-actin (1:5000; Sigma) or Lamin B1 (1:1000; Cell Signaling) antibody as an internal control.

Isolation of airway smooth muscle cells (ASMCs)

ASMCs (passages 3–6) were isolated and cultured as previously described, with slight modifications.⁶ The main bronchial airway segments of mice from each experimental group were isolated and digested with 0.2% collagenase D (Roche, Indianapolis, IN, USA) and 0.05% elastase (Sigma) for 30 min at 37°C in a shaking incubator. After centrifugation, the pellet containing ASMCs was resuspended in Dulbecco's modified Eagle's medium (DMEM/F12) containing 10% fetal bovine serum (Sigma) to stop the reaction.

Intracellular Ca²⁺ measurements

Intracellular Ca²⁺ levels were determined using the Ca²⁺-sensitive fluorescent indicator Fluo-3 AM, as previously described.⁷ ASMCs were incubated with a physiological solution (125 mM NaCl, 3 mM KCl, 1 mM MgCl₂, 10 mM HEPES, and 5 mM glucose, pH 7.4) containing 5 μM Fluo 3-AM (Molecular probe, Eugene, OR, USA) at 37°C for 45 min. The ASMCs were then washed thrice with PBS. Changes in intracellular Ca²⁺ levels were scanned every 5 sec using a confocal laser scanning microscope (Olympus) with emission and excitation at 530 and 488 nm, respectively. In each cell studied, fluorescence intensity (FI, arbitrary units) was measured, and intracellular Ca²⁺ concentration was represented as FI. Normalized FI was calculated as FI divided by the basal FI before histamine treatment (FI₀) to control for variations in basal

fluorescence (FI/FI0). Net changes in FI were represented as (FImax–FI0)/FI0. FImax was the maximum level of FI, which occurred after the addition of histamine.

Gel contraction assay

Collagen contraction assay was performed using a contraction assay kit (Cell Biolabs, Inc., San Diego, CA, USA) as reported.⁷ In brief, ASMCs (1×10^6 cells/mL) were resuspended in culture media and the cell suspension was mixed with collagen (bovine type I) gel working solution. The cell–collagen mixture (0.5 mL) was placed in a 24-well plate and incubated for 1 h at 37°C. After polymerization, 1 mL of the complete medium was added to each well and incubated for 48 h. The cells were treated with histamine (100 μ M) or 2, 3-butanedione monoxime (BDM; 10 mM) 1 h prior to mechanically being loosened from the wells' sides. BDM, a myosin inhibitor, was used as a contraction inhibitor. Gels were imaged using a camera (Samsung Electronics Co., Ltd., Korea), and the size of the gel area was analyzed using ImageJ software (NIH).

Statistics

Data were represented as means $\pm$ SEM. Experimental groups were compared using one-way ANOVA. $p < 0.05$ indicates statistical significance.

References

1. Ryu JH, Xie C, Kim E-J, et al. Reduction of Asthmatic Parameters by Sea Hare Hydrolysates in a Mouse Model of Allergic Asthma. *Nutrients*. 2017;9:699.
2. Myou S, Leff AR, Myo S, et al. Blockade of inflammation and airway hyperresponsiveness in immune-sensitized mice by dominant-negative phosphoinositide 3-kinase–TAT. *J Exp Med*. 2003;198:1573-1582.
3. Ford JG, Rennick D, Donaldson DD, et al. IL-13 and IFN- γ : interactions in lung inflammation. *J Immunol*. 2001;167:1769-1777.
4. Di Valentin E, Crahay C, Garbacki N, et al. New asthma biomarkers: lessons from murine models of acute and chronic asthma. *Am J Physiol Renal Physiol*. 2009;296:L185-L197.
5. Cho JY, Miller M, Baek KJ, et al. Immunostimulatory DNA inhibits transforming growth factor- β expression and airway remodeling. *Am J Respir Cell Mol Biol*. 2004;30:651-661.
6. Wang Q, Li H, Yao Y, et al. Hb-egf–promoted airway smooth muscle cells and their progenitor migration contribute to airway smooth muscle remodeling in asthmatic mouse. *J Immunol*. 2016;196:2361-2367.
7. Yoon CH, Park H-J, Cho Y-W, et al. Cigarette smoke extract-induced reduction in migration and contraction in normal human bronchial smooth muscle cells. *The Korean Journal of Physiology & Pharmacology*. 2011;15:397-403.

Figure legends

Figure S1. The appearance and technical characteristics of the skin-adherent LED-based device.

An LED-based device (width × length × height; 4.9 × 4.9 × 1.3 cm) was used for L-PBM with the following parameters: wavelength, 610 nm [full width at half maximum, 24 nm (orange color)]; irradiance, 1.7 mW/cm²; radiant exposure, 2.0 J/cm²; surface area of electrodes, 1.6 cm; and spot size of electrodes, 0.4 cm diameter.

Figure S2. No effects of sham-L-PBM on asthmatic parameters (n=4).

(A) AHR to methacholine challenge. (B) Total and (C) differential cell counts in BALF. (D) Representative images of H&E and (E) PAS staining of the lung tissues. Scale bar=50 μm. [†] *p* < 0.05, compared to PBS + L-PBM. [‡] *p* < 0.05, compared to OVA. [§] *p* < 0.05, compared to OVA + sham-L-PBM.

Abbreviations: L-PBM, light-emitting diodes-based photobiomodulation; H&E, hematoxylin and eosin; PAS, periodic acid–Schiff.

Figure S3. No alterations in the size of immune organs by L-PBM.

(A) Representative images of the thymus and spleen isolated from mice. Scale bar=1 cm. (B) Representative images of H&E staining of the thymus and spleen. Scale bar=0.5 cm. (C) The ratios of organ weight to body weight were calculated (n=10). [†] *p* < 0.05, compared to PBS + L-PBM. [‡] *p* < 0.05, compared to OVA. [§] *p* < 0.05, compared to OVA + DEX.

Figure S4. Inhibitory effect of L-PBM on phosphorylation of MAPKs and NF-κB activation in lung tissues obtained from asthmatic mice.

(A) Reduction in levels of p-ERK, p-p38, and p-JNK by L-PBM. (B) Suppression of OVA-induced NF-κB nuclear translocation by L-PBM (n=4). [†] *p* < 0.05, compared to PBS + L-PBM. [‡] *p* < 0.05, compared to OVA.

Abbreviations: MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase