Therapeutic Effects of Artificial Light Therapy on Allergic Bronchial Asthma in a Mouse Model

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Abstract

Background: Asthma is a chronic inflammatory airways disorder, which is affected by a gene and environmental factors, and influences on 300 million people in the world and causes 25,000 human deaths every year. The mechanism of pathogenesis of asthma has been still not cleared, and the certain precaution has not been found yet. Recently, sunlight has been reported to improve symptoms of asthma. We recently found that irradiation with artificial light suppresses symptoms or inflammation in animal models of inflammatory bowel disease or NASH (non-alcoholic steatohepatitis).

Objective: To evaluate the effects of irradiation with artificial light with a spectrum approximating that of sunlight on a mouse model of ovalbumin-induced asthma and its mechanism of action.

Materials and Methods: Mice were divided into three groups: Control group, mice without ovalbumin-induced asthma that were bred under normal fluorescent light; Asthma group, mice with ovalbumin-induced asthma that were bred under artificial light. Asthma was induced by intraperitoneal injection of ovalbumin, followed by intranasal administration of ovalbumin. At the end of the experiment, histopathological findings of lung, concentrations of Immunoglobulin E antibody and cytokines (tumor necrosis factor [TNF]-\(\alpha\), interleukin [IL]-6, IL-13, and IL-17) in serum or broncho-alveolar lavage fluid (BALF) as well as expression of mitochondrial uncoupling protein (UCP)2 in BALF were investigated.

Results: Histological findings of lung in the Asthma group revealed bronchial wall thickening and invasion of inflammatory cells, whereas these changes were only slightly found in the Asthma + light group. Compared with the Asthma group, serum concentration of IgE antibody in the Asthma + light group was significantly suppressed (\(P < 0.05\)). Concentrations of TNF-\(\alpha\) and IL-13 in BALF and serum concentrations of IL-6 and IL-17 were more significantly suppressed in the Asthma + light group than in the Asthma group (all, \(P < 0.05\)). Compared with that in the Asthma and Control groups, the expression of UCP2 in the Asthma + light group was significantly increased (\(P < 0.05\) for both).

Conclusion: Irradiation with artificial light appeared to have a suppressive effect on asthma through suppressive effects on serum IgE antibody and inflammatory cytokines in a mouse model of asthma.

Keywords: Phototherapy; Asthma; Immunoglobulin E; IL-13; UCP2

Introduction

Asthma affects 300 million people globally and causes 25,000 deaths annually [1]. The mechanism of pathogenesis of asthma has been still not cleared, and the certain precaution has not been found yet [1]. It has recently become obvious that sunlight effects improvement on various diseases. Sunbathing is reported to help improve allergic or autoimmune diseases [2].

Sunlight reportedly has an anti-inflammatory ability to facilitate excretion of inflammatory substances or an ability to promote repair and regeneration of damaged tissues [3]. However, changes in lifestyle or working style and anxiety regarding the risk for sunburn or skin cancer caused by ultraviolet rays have presently decreased the indoor lifestyle or working style and anxiety regarding the risk for sunburn and regeneration of damaged tissues [3]. Additionally, patients who require hospitalization or recuperation at home are confined to an indoor lifestyle. We recently found that irradiation from commercially available lamps that emit artificial light suppresses symptoms or inflammation in animal models of inflammatory bowel disease and NASH (non-alcoholic steatohepatitis) [5,6]. The objectives of this study were to evaluate whether irradiation from commercially available artificial light with a wavelength similar to that of sunlight suppresses asthma in a mouse model of ovalbumin-induced asthma and to clarify the effects of artificial light on serum concentration of Immunoglobulin E (IgE) and concentrations of cytokines in serum or broncho-alveolar lavage fluid (BALF) and on the level of expression of UCP2 in the lung tissues to reveal the mechanism of action of artificial light.

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Material and Methods

Animals

Six-week-old male NC/Nga mice (Japan SLC, Shizuoka, Japan) were maintained at 21°C on a 12-h light-dark cycle and given free access to water and standard laboratory chow.

Establishment of a mouse model of asthma

Mice were intraperitoneally injected with 100μl of saline containing 250μg of ovalbumin (Sigma-Aldrich Japan Co., Tokyo, Japan) absorbed in 50μl of aluminum hydroxide dried gel 99% (Alum; Strem Chemicals, Inc., Newburyport, MA, USA). This immunization with ovalbumin/alum (Strem Chemicals Inc., Bischheim, France) was conducted in mice with two injections administered on day 7 and day 14 of the experimental period. Asthma was then triggered by intranasal administration of 20μl of PBS containing 1 mg of ovalbumin every day from day 7 until day 30 in the ovalbumin-immunized mice by means of the modified method of Iwasaki et al. [7].

Phototherapy

To investigate the therapeutic effects of phototherapy on asthma, mice were randomly divided into three groups: Control group, Asthma group, and Asthma + light group. Artificial light (color temperature 5500 K, color rendition index > 90; Chang Gung Biotechnology, Taipei, Taiwan), which was used in our previous study, was also used for the Asthma + light group. Normal fluorescent lamps (color temperature 4500 K, color rendition index < 70; National, Japan) were used for the Control and Asthma groups. Each lamp illuminated the mice for 12 h/day. Light intensity in the cages of the Asthma + light group was maintained at 1000 lux, and that for the Control and Asthma groups was maintained at 200 lux with a luminaire (CL-200A, Konica Minolta, Japan). Light intensity in the cages of the Control and Asthma groups was maintained at a power intensity of 63μW/cm² and a daily energy density of 2.76 J/cm². Treatment consisted of daily irradiation with artificial light in the Asthma + light group for 12 h at a power intensity of 333μW/cm² and daily energy density of 14.45 J/cm².

All mice were bred in an environment of normal fluorescent light or artificial light from day 0 of the experimental period until day30. The experimental murine breeding shelf used in our experiments comprised a screen to cut out external light and a ventilator to maintain a cage temperature of 21°C. One cage was used to breed one mouse. All mice were euthanized on day 30 at 6 hours after a final intranasal administration of PBS containing ovalbumin. BALF, endocardial blood, and lung specimens were obtained from the euthanized mice. BALF was collected by washing the lungs of euthanized mice. Histological evaluation was performed in a blinded fashion by two researchers who had no knowledge of the group to which each mouse belonged.

Analysis of serum immunoglobulin E (IgE)

The concentration of murine serum IgE was determined using a sandwich enzyme-linked immunosorbent assay (Acris Antibodies GmbH, Herford, Germany) according to the manufacturer’s instructions. Absorption at 450 nm was read on a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Analysis of serum cytokines and chemokines

As we described previously, [6] a multiplex sandwich immunoassay, which contains fluorescence-labeled microspheres conjugated with monoclonal antibodies, was performed with a Bio-Plex protein array system (Bio-Rad Laboratories, Hercules, CA, USA). Serum samples and BALF samples were thawed and run in duplicate. Antibody-coupled beads were incubated with serum samples, incubated with biotinylated secondary (detection) antibody, and finally, incubated with streptavidin-phycocerythrin. Bound molecules were then read by the Bio-Plex array reader, which uses LumineX fluorescent bead-based technology (LumineX, Austin, TX, USA) and allows multiple measurements of the sample, resulting in the effective quantitation of cytokines. Analyses performed included those of interleukin TNF-α, interleukin (IL)-13, IL-6, and IL-17. All antibodies were purchased from Invitrogen Corporation (Carlsbad, CA, USA).

Analysis of lung uncoupling protein 2 (UCP2) levels

The levels of UCP2 in lung homogenates were determined using a Western blot analysis. Lysates were added to 2-mercaptoethanol and boiled for 5 minutes at 100°C. Proteins (75μg/sample) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Membranes were blocked with 1% non-fat dry milk in Tris-buffered saline (TBS) with 0.1% Tween-20 and incubated with primary antibodies against UCP2 and beta-actin (both from Abcam PLC, Cambridge, UK) for 24 hours at 4°C in a refrigerator. The membranes were washed with 0.1% Tween 20 in TBS and then incubated with anti-mouse and anti-rabbit secondary antibodies (Immunobio-Logical Laboratories Co., Fujioka city, Japan) at room temperature for 60 minutes. Proteins were visualized with ECL Western blot analysis detection reagent (GE Healthcare UK Ltd., Buckinghamshire, UK). Images were analyzed with Image J software (NIH Image, Bethesda, MD).

Statistical analysis

Data are shown as mean ± SE (standard error). Data were analyzed by Mann-Whitney U test for single comparisons. A value of P < 0.05 was considered to be statistically significant. Stat-View Ver. 5.0 software (Abacus Concepts Inc., Berkeley, CA, USA) was used for all statistical analyses.

Ethical considerations

The study protocol was approved by the animal ethics review committee of Oita University, Faculty of Medicine.

Results
Histological evaluation of the lung

Both hyperplasia of the respiratory epithelium and the invasion of inflammatory cells were more remarkable in the Asthma group than Control group, whereas both were decreased in the Asthma + light group as compared with the Asthma group (Figure 1).

Analysis of serum IgE

Serum IgE concentration in the Asthma group was significantly increased compared with that in the Control group (1793.5 ± 117.3 vs.149.5 ± 28 ng/ml, P = 0.008). Serum IgE concentration in the Asthma + light group was significantly decreased compared with that in the Asthma group (1235.3 ± 88 vs. 1793.5 ± 117.3 ng/ml, P = 0.0033) (Figure 2).

Concentrations of TNF-α and IL-13 in the BALF

Concentrations of TNF-α and IL-13 in the BALF in the Asthma group were significantly higher than those in the Control group. (2443.6 ± 754.6 vs. 27.5 ± 2.5 ng/ml, P = 0.0008; 178.8 ± 24.7 vs. 28.2 ± 6.8 ng/ml, P = 0.0008, respectively). Concentrations of TNF-α and IL-13 in the Asthma + light group were significantly lower than those in the Asthma group (645.7 ± 348.8 vs. 2443.6 ± 754.6 ng/ml, P = 0.0209; 48.8 ± 12.1 vs. 178.8 ± 24.7 ng/ml, P = 0.0023, respectively). The concentrations of TNF-α and IL-13 in the Asthma + light group were not significantly different compared with those in the Control group (Figure 3a and 3b).

Concentrations of IL-6 and IL-17 in the serum

Concentrations of serum IL-6 and IL-17 in the Asthma group were significantly higher than those in the Control group (41.5 ± 8.5 vs. 12.7 ± 1.4 pg/ml, P = 0.0026; 22.6 ± 6.3 vs. 5.5 ± 1.4 pg/ml, P = 0.0090, respectively). Concentrations of serum IL-6 and IL-17 in the Asthma + light group were significantly lower than those in the Asthma group (17.7 ± 3.6 vs. 41.5 ± 8.5 pg/ml, P = 0.0098; 6.7 ± 1.2 vs. 22.6 ± 6.3 pg/ml, P = 0.0455, respectively) (Figure 4a and 4b).

Analysis of lung UCP2 levels

The expression of UCP2 in the Asthma group was significantly increased compared with that in the Control group (11.5 ± 0.25 vs. 10.1 ± 0.25 pg/ml, P = 0.0495). The expression of UCP2 in the Asthma + light group was significantly increased over that in the Asthma group (15.7 ± 1.2 vs. 11.5 ± 0.25 pg/ml, P = 0.0495) (Figure 5a and 5b).

Discussion

This study is the first report, to our knowledge, that irradiation...
with artificial light to the body surface suppressed asthma in a mouse model of ovalbumin-induced asthma. Although ultraviolet irradiation has been reported to improve ovalbumin-induced asthma, ultraviolet B8-10 carries the risk of carcinogenesis. The light used in the present study is the light of commercially available household fluorescent lamps. The mice with ovalbumin-induced asthma are similar to humans in terms of histopathological findings and the mechanism of action and kinetics of cytokines, and they are almost universally used as the mouse model of asthma [7].

The levels of IL-6 and IL-17 in serum increase in both humans with asthma and mice with ovalbumin-induced asthma, and they play an important role in the pathological process of asthma [11,12]. IL-6 can be to exacerbate lung pathology by its effect on IL-17 and neutrophil accumulation. IL-17 has been shown to trigger the production of IL-6 by lung epithelial cells [12]. The results of our study showed a decrease in invasion of inflammatory cells in the lung and a decrease in airway epithelium thickening and suppression of serum IL-6 and IL-17 in this mouse model of asthma irradiated with artificial light. Invasion of inflammatory cells in the lung and airway epithelium thickening are the histopathological findings found in the lungs of patients with asthma. IL-13 and TNF-α in BALF promote induction of the invasion of inflammatory cells from airway epithelium and accelerate hyper permeability of eosinophils in the vascular endothelial cells [13].

A study in which irradiation with artificial light decreased the levels of IL-13 and TNF-α in BALF supports the histopathological findings of the lung in the present study [14]. Accordingly, irradiation with artificial light possibly suppressed asthma by affecting the whole body of the mice. In addition, irradiation with artificial light showed suppressive effects on serum IgE and IL-13 as well as on TNF-α in BALF. IgE antibody is one of the major factors involved in the onset of asthma and is generated by B cells via stimulation of IL-4, which is generated by Th2 cells, resulting from a response to an allergen [15]. The IgE antibody-allergen complex then induces the generation of IL-13 and TNF-α that are inflammatory cytokines in mast cells [16]. Therefore, irradiation with artificial light demonstrates the potential for suppressive effects on asthma by directly or indirectly suppressing IgE antibody generation of B cells. UCP, however, is a mitochondrial uncoupling protein that is associated with autonomous control over internal energy consumption.

UCP2 is also generated by such immune cells as macrophages or mast cells and reportedly has the potential for playing roles in immune regulation and anti-inflammatory activity [17]. Also, UCP2 have a protective effect of B cells against oxidative stress [18]. Compared to that in healthy individuals, the level of oxidative stress in patients with asthma is significantly higher [19]. In particular, UCP2 is related to control of the generation of chemical mediators in mast cells [20]. In this study, UCP2 increased more significantly in the Asthma + light group than in the Asthma group, suggesting that activation of UCP2 by artificial light therapy is associated with
an anti-inflammatory or a suppressive effect on degranulation of mast cells. In other words, light irradiation has the possibility of expressing effects through mechanisms of action by mitochondria in cells.

Furthermore, IL-13 has an effect to promote cell remodeling by fibers in the airway and airway remodeling. Thus, irradiation with artificial light possibly suppresses airway remodeling that is an irreversible change of asthma. The effects on the improvement of asthma by suppression of IL-13 were demonstrated in the clinical study of lebrikizumab, a monoclonal antibody to interleukin-13, with results showing that inhaled corticosteroid significantly improved respiratory function in patients with poorly controlled asthma [21].

However, these drugs have adverse effects. In our study, on the contrary, irradiation with artificial light has no apparent adverse effects and has preventive effects on remodeling, leading to its immediate clinical application, which is thought to be a novel finding.

This study has some limitations. First, specified wavelengths of ultraviolet rays, far-infrared irradiation, or visible irradiation that are included in the artificial light used in this study could be associated with the expression of effects. Second, it is unclear which intensity of light is most effective for treatment. Further studies are required to clarify these points. In conclusion, our study demonstrated that irradiation with artificial light of the body surface showed suppressed effects on serum IgE antibody and inflammatory cytokines and promoted UCP2 expression, resulting in suppression of asthma.

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References