Non-thermal plasma inhibits mast cell activation and ameliorates allergic skin inflammatory diseases in NC/Nga mice.

Myung-Hoon Lee[1,2*], Yun Sang Lee[1*], Haeng Jun Kim[1,2], Chang Hak Han[1,2], Sung Un Kang[1] and Chul-Ho Kim[1,2,]

[1]*Department of Otolaryngology, School of Medicine, Ajou University, Suwon, Republic of Korea,*

[2]*Department of Molecular Science and Technology, Ajou University, Suwon, Republic of Korea,*

Abstract: Non-thermal plasma (NTP) has many functional activities. Despite of its wide spread biomedical application, the effect of NTP on immune cells and allergic response has not been well studied. In this study, we determined whether NTP suppresses mast cell activation, which is important for allergic response, and ameliorates an atopic dermatitis (AD)-like skin inflammatory disease in mice.

Keywords: Non-thermal plasma, mast cell, atopic dermatitis, NC/Nga mice.

1. Introduction

Atopic dermatitis (AD) is a common allergic skin disease, characterized by mast cell activation, eosinophilia, overexpression of cytokines and epithelial hyperplasia [1,2]. Although the etiology of AD is complex, many studies suggest that immune cells are involved in the pathogenesis of AD and uncontrolled immune response is one of the main causes of AD [3]. For example, in the acute phase of AD, Th2 cell percentage increases, and AD skin lesions express higher levels of Th2 cytokines and chemokines compared to normal skin [4,5]. In addition, the activation and infiltration of mast cells and eosinophils are also critical for AD [6]. By contrast, Th1 cells and their cytokines play a role in the chronic phase of AD [7]. Several treatments for AD, such as glucocorticoids, calcineurin inhibitors, phototherapy, and immune-suppressors (cyclosporine A), have been used [8]. However, these drugs and therapies cause many side effects including ulcers, thin skin, diabetes, depression, and slow-wound healing [9]. Therefore, the development of new treatments for AD without side effects imperative. Plasma is referred to as the fourth state of matter and is composed of cations, anions, electrons and reactive species. During the last few decades, the field of plasma medicine has grown rapidly. Several studies show that plasma regulates various effects, such as anti-cancer [10-13], anti-inflammation [14], sterilization [15], and tooth-bleaching effects [16]. Recently, we demonstrated that non-thermal plasma (NTP) treatment induces cancer cell death via AKT degradation [17], promotes muscle regeneration in mice [18], accelerates wound healing [19], and inhibits psoriasis-like skin inflammation in mice [20]. Thus, plasma medicine is emerging in the biomedical field, and researchers are investigating whether plasma can be used for the treatment of various diseases. However, the anti-allergic effect of NTP is not well studied. In this study, we investigated whether NTP inhibited mast cell activation and house dust mite (HDM)-induced AD-like skin inflammation in NC/Nga mice.

2. Materials and Methods

LTP generation and treatment

LTP was generated by the treatment of non-thermal N_2 plasma into culture media (RPMI 1640 or DMEM) for 60sec per ml at a 2cm distant from the media (Fig. 1B). Cells were treated with LTP for 6 h for real-time PCR analysis. HaCaT cells were incubated with LTP for 15 min for pSTAT6, STAT6, pNF-κB and NF-κB detection.

Cell line and reagents

The immortalized human keratinocyte cell line, HaCaT was grown as monolayer cultures in DMEM (Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (Gibco, Carlsbad CA), 1% penicillin and streptomycin (Gibco). Antibodies for STAT6, p-STAT6, NF-κB, p-NF-κB and GAPDH were purchased from Cell signaling technology (Danvers, MA). Anti-eosinophil peroxidase antibodies were purchased from Santa Cruz biotechnology. All recombinant cytokines were purchased from Peprotech (Rocky Hill, NJ).

RNA isolation, complementary DNA synthesis, and quantitative real-time PCR analysis.

Total RNA was isolated from cells or mouse skin tissue using TRIzol® reagent (GIBCOBRL, Grand Island, NY, USA). The first strand of cDNA was synthesized form 1μg of total RNA using a reverse transcription system (TOYOBO, Japan). The primer sets for real-time PCR analysis were purchased from Qiagen. GAPDH mRNA was used as an endogenous control. The amplification program consisted of 1 cycle at 95 °C for 10 min, followed by 45 cycles at 95 °C for 20sec, 55 °C for 20 sec, and 72 for 20 sec.

Western blot analysis.

Western blot analysis was performed as previously described with minor modification [21]. Cells were lysed in RIPA buffer (Sigma Aldrich, St. Louis, MO, USA) containing a protease inhibitor cocktail and PhoSTOP (Roche Molecular Biochemicals, Basel, Switzerland). Ten micrograms of the protein lysate were resolved by SDS/PAGE (10% gel) and transferred to PVDF membranes (Amersham, Arlington Heights, IL, USA). Upon the completion of transfer, the membranes were probed with appropriate antibodies. The bands were visualized with Advanced ECL® Western Blotting Detection Reagents (Amersham). Equal protein loading was ensured using an anti-GAPDH antibody (Cell Signaling Technology).

Mice

Six-week-old male NC/Nga mice were obtained from Orient Bio (Korea). These mice were housed in a controlled room with a 12:12-hour light-dark cycle and free

access to laboratory chow and water. Mice between 7-9 weeks of age were used. The animal experiments in this study were approved by the Committee for Ethics in Animal Experiment of Ajou University School of Medicine and performed in accordance with the institution guidelines.

HDM-induced AD in mice

AD was induced in NC/Nga mice by application of HDM as described previously with minor modification [22]. Briefly, dorsal hairs of the mice were removed completely. Next day, 200µl of 4% SDS solution were used to dorsal skin to break the skin barrier followed by application of 100 mg of HDM containing cream (Biostir) every 4 days for 3 weeks. All the mice were sacrificed at a day after the final treatment. The mice were divided into four groups: non-treated control, NTP-treated only, Biostir-treated only and the topical application of NTP to Biostir-treated mice.

Generation of mouse bone marrow-derived mast cells

BMMC were generated as previously described with minor modification [23]. Briefly mouse lineage-negative cells were isolated from the bone marrow cells using the lineage-negative cell isolation kit (Miltenyl Biotech, Germany) according to the manufacturer's protocol. The lineage-negative cells were cultured in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in the presence of mIL-3 (10ng/ml) and mSCF (50ng/ml) for 8 weeks at 37 °C in a humidified atmosphere with 5% CO_2. The mast cell differentiation was determined by FACS analysis, and > 94 % of the cells were positive for CD117 (c-Kit) and FcεRI.

Statistical analysis

Statistical comparisons between groups were made using unpaired two-sided t-tests and difference with < 0.05 (*), < 0.01 (**) and < 0.001 (***) were considered significant. All experiments were performed at least three times.

3. Results

LTP treatment inhibits mast cell activation

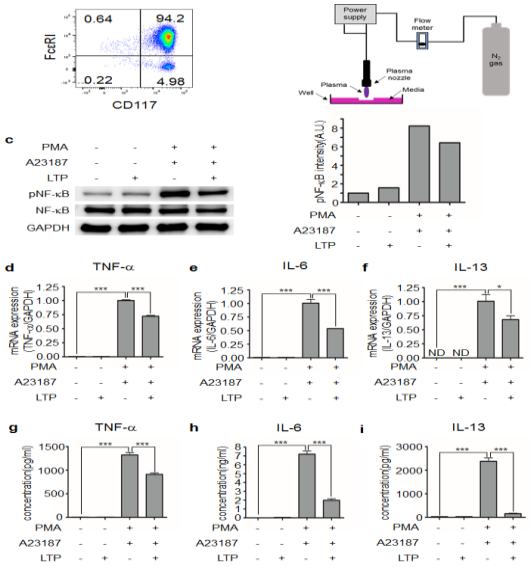

Fig. 1. LTP inhibits mast cell activation. (A) Mast cells were generated from mouse bone-marrow derived lineage negative cells by culture with mIL-3 and mSCF for 8 weeks. The percentage of mast cells exceed 94%. (B) A schematic diagram showing the generation of LTP. (C) LTP treatment inhibits NF-κB activation in PMA/A23187-stimulated mast cells. LTP treatment inhibits (D) TNF-α, (E) IL-6, and (F) IL-13 expression in PMA/A23187-stimulated mast cells. LTP treatment reduced the secretion of (G) TNF-α, (H) IL-6, and (I) IL-13 by activated mast cells. *P <0.05, **P <0.01, ***P <0.001.

NTP treatment ameliorate HDM-induced AD-like skin inflammation in mice.

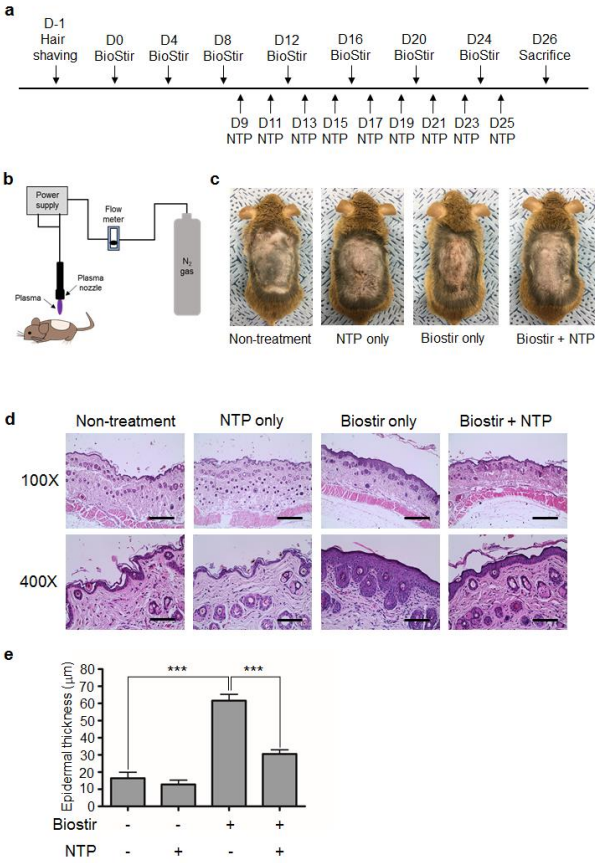

Fig. 2. NTP treatment ameliorates HDM-induced AD-like skin inflammation in NC/Nga mice. (A) The experimental scheme for induction of HDM-induced AD-like skin inflammation and NTP treatment. (B) A schematic diagram describing treatment of mice with the NTP- producing machine. (C) NTP treatment inhibited AD-like skin inflammation in NC/Nga mice. (D) H&E staining of mouse back skin (E) NTP treatment reduced epidermal thickness. **P <0.01, ***P <0.001. Bar = 400 µm (100X), 100 µm (400X).

NTP treatment inhibits immune cell infiltration in the mouse skin.

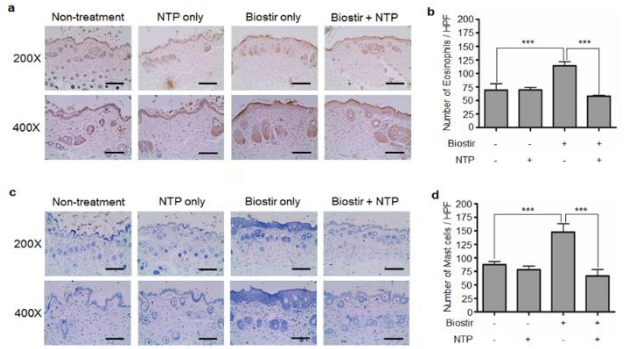

Fig. 3. NTP treatment inhibits immune cell infiltration in the mouse skin. (A) NTP treatment inhibited eosinophil infiltration in HDM-induced AD-like mouse skin. (B) The number of infiltrated eosinophils is indicated as a bar graph. (C) NTP treatment inhibited mast cell infiltration in HDM-induced AD-like mouse skin. (D) The number of infiltrated mast cells is represented as a bar graph. *P <0.05, **P <0.01. Bar = 100 μm.

NTP treatment inhibits Th2 cell differentiation and AD-related gene expression in mice.

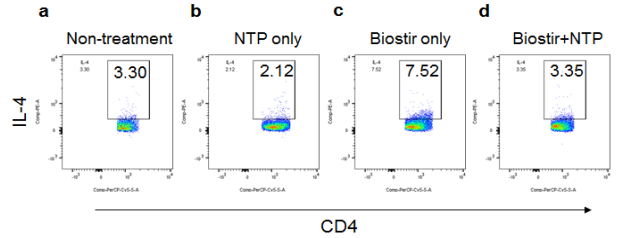

Fig. 4. NTP treatment inhibited Th2 cell differentiation *in vivo*. Th2 cell differentiation in the draining lymph node in (A) non-treated and (B) NTP only-treated mice was similar. (C) HDM (Biostir)-induced Th2 cell differentiation in the mouse draining lymph node. (D) NTP treatment inhibited HDM-induced Th2 cell differentiation.

NTP treatment suppressed AD-related inflammatory cytokine and chemokine expression in the mouse skin.

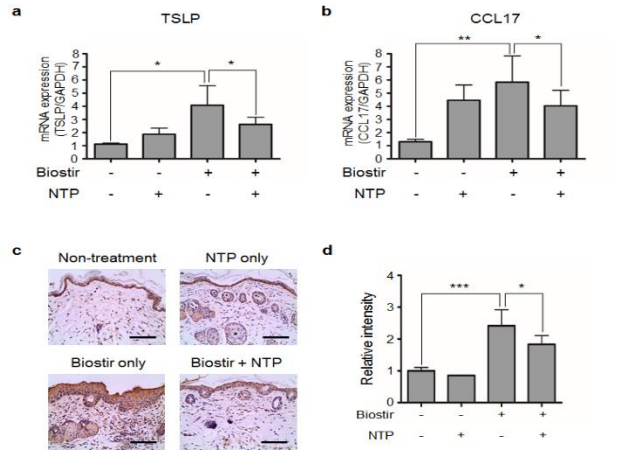

Fig. 5. Cytokine and chemokine expression in the mouse skin. AD-related cytokine. (A) TSLP and (B) chemokine CCL17 expression increased in HDM-applied mouse skin and the expression was inhibited by NTP treatment. (C) NTP treatment reduced HDM-induced TSLP production in mouse skin. TSLP production was detected by IHC. (D) The level of TSLP is shown as a bar graph. *P <0.05, **P <0.01. Bar = 100 μm.

LTP treatment inhibits the inflammatory response in HaCaT.

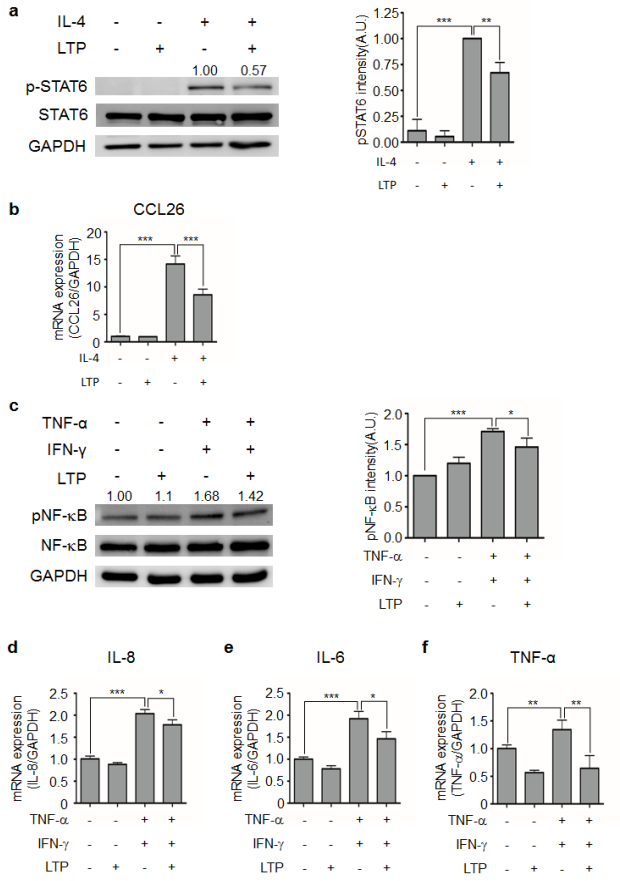

Fig.6. LTP treatment inhibits allergic inflammatory responses in HaCaT. (A) All Western blotting experiments were performed under the same condition. LTP treatment inhibited IL-4-induced STAT6 activation in HaCaT. IL-4 was stimulated with or without LTP for 15 min and the cells were collected for p-STAT6 detection using western blot analysis. The level of p-STAT6 was measured using Image J software. (B) LTP treatment inhibited IL-4-induced CCL26 expression in HaCaT. (C) All Western blotting experiments were performed under the same condition. LTP treatment inhibited TNF-α/IFN-γ-induced NF-κB activation in HaCaT. HaCaT cells were stimulated with TNF-α and IFN-γ for 15 min and the cells were harvested for p-NF-κB level using western blot analysis. The level of p-NF-κB was measured using Image J software. The expression of pro-inflammatory chemokine, (D) IL-8, and cytokine, (E) IL-6 and (F) TNF-α was detected in TNF-α/IFN-γ-stimulated HaCaT with or without LTP treatment using real-time PCR analysis. *P <0.05, **P <0.01, ***P <0.001.

4. Conclusion

In this study, we demonstrated that NTP can ameliorate AD-like skin inflammation in a mouse model of AD induced with HDM in NC/Nga mice. These results might suggest novel applications of NTP for the treatment of AD and other allergic diseases.

5. References

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