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Effect of a Temperature-Adjustable Cryotherapy Device on Mice with Lysophosphatidic Acid-Induced Pruritus

Mi Hee Kwack[1], Chang Hyun Song, Seongjin Lee[2], Gi Ung Ha, Gun-Ho Kim[2], Weon Ju Lee

Department of Dermatology, School of Medicine, Kyungpook National University, Daegu, Korea; [1]Department of Immunology and BK21 FOUR KNU Convergence Educational Program of Biomedical Sciences for Creative Future Talents, School of Medicine, Kyungpook National University, Daegu, Korea; [2]Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea

Dear Editor:

Pruritus impacts daytime activities as well as affected individual's psychosocial health[1]. Various nervous system mediators, such as transient receptor potential (TRP) ion channels, are associated with the chronic pruritus mechanism[2]. Particularly, several TRP ion channels, such as transient receptor potential melastatin 8 (TRPM8), play a regulatory role in thermal changes of skin, which reportedly modulate itch sensitivity[3]. Lysophosphatidic acid (LPA), a pruritus mediator and a member of the lysophospholipid family, activates transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential vanilloid 1 (TRPV1)[4].

A novel cryotherapy device which can accurately cool a target area from –20℃ to 10℃ was newly developed and provided by RecensMedical Inc (Fig. 1A, B). This system has the ability to regulate the thermodynamic state (i.e., temperature and pressure) of cryogenic substances, calculate the difference between the target temperature and the set temperature.

After we checked with real-time polymerase chain reaction (PCR) that itch response of mice to histamine is weaker than expected (Fig. 2A), LPA was selected for this study. LPA (3 μM) was intradermally injected at two points on the back of female 7-week-old HR-1 mice, in a 10 μl solution. Mild reddish macules occurred at the LPA-injected sites. After 1 week of the injection, they were treated with the novel cryotherapy system with the accurate temperature of either –5℃, 0℃ or 5℃ for 5, 10, and 20 sec (Fig. 1C). Itch-related biomarkers' mRNA and protein expression were estimated by real-time PCR and immunohistochemistry. The Institutional Animal Care and Use Committee of Kyungpook National University (No. 2018-0167) approved this study.

TRIzol reagent was used to extract the total RNA. Subsequently, cDNA was compounded. In real-time PCR, specific oligonucleotide primers for TRPA1, TRPV1, TRPM8, protease activated receptor 2 (PAR2), interleukin (IL)-4, IL-10, IL-13, IL-31, and interferon (IFN)-γ were used. The PCR primers used in this study are summarized in Supplementary Table 1.

From the mice, we gained tissue samples and then they were placed in cryomolds with embedding medium. After 1 hour of preparation with 5% normal donkey serum, the samples were incubated overnight at 4℃. During the incubation, antibodies for TRPA1, TRPV1, TRPM8, PAR2, IL-4, IL-10, IL-13, IL-31, and IFN-γ were added. Afterwards, we washed the samples for 3 times using phosphate buffered saline, followed

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Corresponding Author
Weon Ju Lee
Department of Dermatology, Kyungpook National University Hospital, 130 Dongdeok-ro, Jung-gu, Daegu 41944, Korea
Tel: +82-53-420-5838
Fax: +82-53-426-0770
E-mail: weonju@knu.ac.kr
https://orcid.org/0000-0001-5708-1305

Gun-Ho Kim
Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, 50 UNIST-gil, Eonyang-eup, Ulju-gun, Ulsan 44919, Korea
Tel: +82-52-217-2413
Fax: +82-52-217-1289
E-mail: gunhokim@unist.ac.kr
https://orcid.org/0000-0002-6020-2590

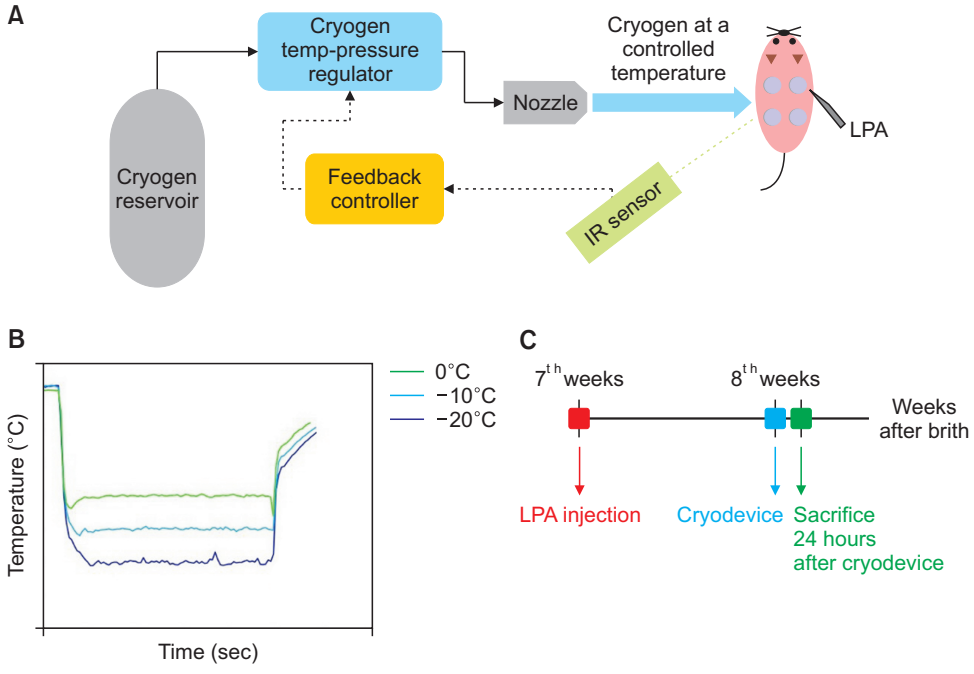

Fig. 1. Novel cryotherapy system that can produce precision cooling temperature at a target area, enabled by feedback control of cryogen thermodynamic state based on real-time temperature reading at the target area. (A) Illustration of the cryotherapy system. (B) Temperature controlled at the target area by the cryotherapy system. (C) Experimental protocol. LPA: lysophosphatidic acid, IR: infrared.

by incubation with donkey anti-rabbit horseradish peroxidase–conjugated antibody for 1 hour. Immunohistochemistry was performed twice.

Gene expression level of the following itch-related biomarkers declined after various conditions of cryotherapy (Fig. 2B): TRPA1 (at 5℃ for 5, 10, and 20 sec; at 0℃ for 5, 10, and 20 sec; and at −5℃ for 5 sec); TRPV1 (at 5℃ for 5, 10, and 20 sec; and at 0℃ for 5, 10, and 20 sec); TRPM8 (at 5℃ for 10 and 20 sec; and at 0℃ for 5, 10, and 20 sec); PAR2 (at 5℃ for 5, 10, and 20 sec; at 0℃ for 5, 10, and 20 sec; and at −5℃ for 5 sec); IL-4, IL-10, IL-13, IL-31, and IFN-γ (all at 5℃ for 5, 10, and 20 sec; at 0℃ for 5, 10, and 20 sec; and at −5℃ for 5, 10, and 20 sec; excluding IL-10 at −5℃ for 20 sec).

Protein expression level of the following itch-related biomarkers declined after various conditions of cryotherapy (Fig. 2C): TRPA1 (at 5℃ for 10 and 20 sec; at 0℃ for 5, 10, and 20 sec; and at −5℃ for 5, 10, and 20 sec); TRPV1 (at 5℃ for 5, 10, and 20 sec; at 0℃ for 5 and 10 sec; and at −5℃ for 5, 10, and 20 sec); TRPM8 (at 5℃ for 20 sec; at 0℃ for 5, 10, and 20 sec; and at −5℃ for 5 sec); PAR2 (at 5℃ for 5, 10, and 20 sec; at 0℃ for 5, 10, and 20 sec; and at −5℃ for 5, 10, and 20 sec); IL-4, IL-10, IL-13, IL-31, and IFN-γ (at 5℃ for 5, 10, and 20 sec; at 0℃ for 5, 10, and 20 sec; and at −5℃ for 5, 10, and 20 sec; excluding IL-4 at 0℃ for 20 sec; IL-10 at −5℃ for 10 sec; and IL-13 at −5℃ for 10 and 20 sec).

Persisting pruritus is one of the characteristic symptoms of atopic dermatitis (AD). Corticosteroids and immunosuppressants have been known and used to relieve pruritus by inhibiting inflammation of AD[5]. However, as these treatment modalities do not target the substantial neural component of the pathophysiology of the pruritus, the treatments are not always successful. Hence, alternative remedies that directly targeting these pathways are required.

Intensity of pruritus is significantly influenced by changes of skin temperature[6]. Particularly, skin cooling can be used as an effective treatment that temporarily relieves pain in various pruritus-causing dermatologic disorders[7]. Skin cooling exerts an anti-pruritic effect by reducing conduction velocity and nerve excitability, as well as slow neuropeptide release and the biochemical mechanisms essential for neurotransmission[8]. Furthermore, release of pruritus-inducing substances is reduced by vasoconstriction of skin induced by cooling[6].

This experiment analyzed various chronic pruritus correlated with chemokines and cytokines. IL-31, in particular, is a T helper 2 cell-derived cytokine that appears to play an essential role in pruritus in patients with AD and is receiving attention as a novel and effective therapeutic target for pruritus[9].

The aim of this study was to estimate the possibility of antipruritic effects of diverse cooling impulse from −5℃ to 5°C by comparing gene and protein expression levels relevant with inflammation and itching. In general, the expression of itch-related biomarkers was downregulated in cold temperatures at the gene and protein levels. The statistically significant

reduction was different for each biomarker according to the thermodynamic state, temperature and duration of treatments. Downregulation of itch-related biomarkers, especially IFN-γ, IL-4, IL-13, and IL-31, at 5℃ for 10 and 20 sec and at 0℃ for 5, 10 sec may be maximized according to this study. In addition, it is found that the expression of TRP channels can

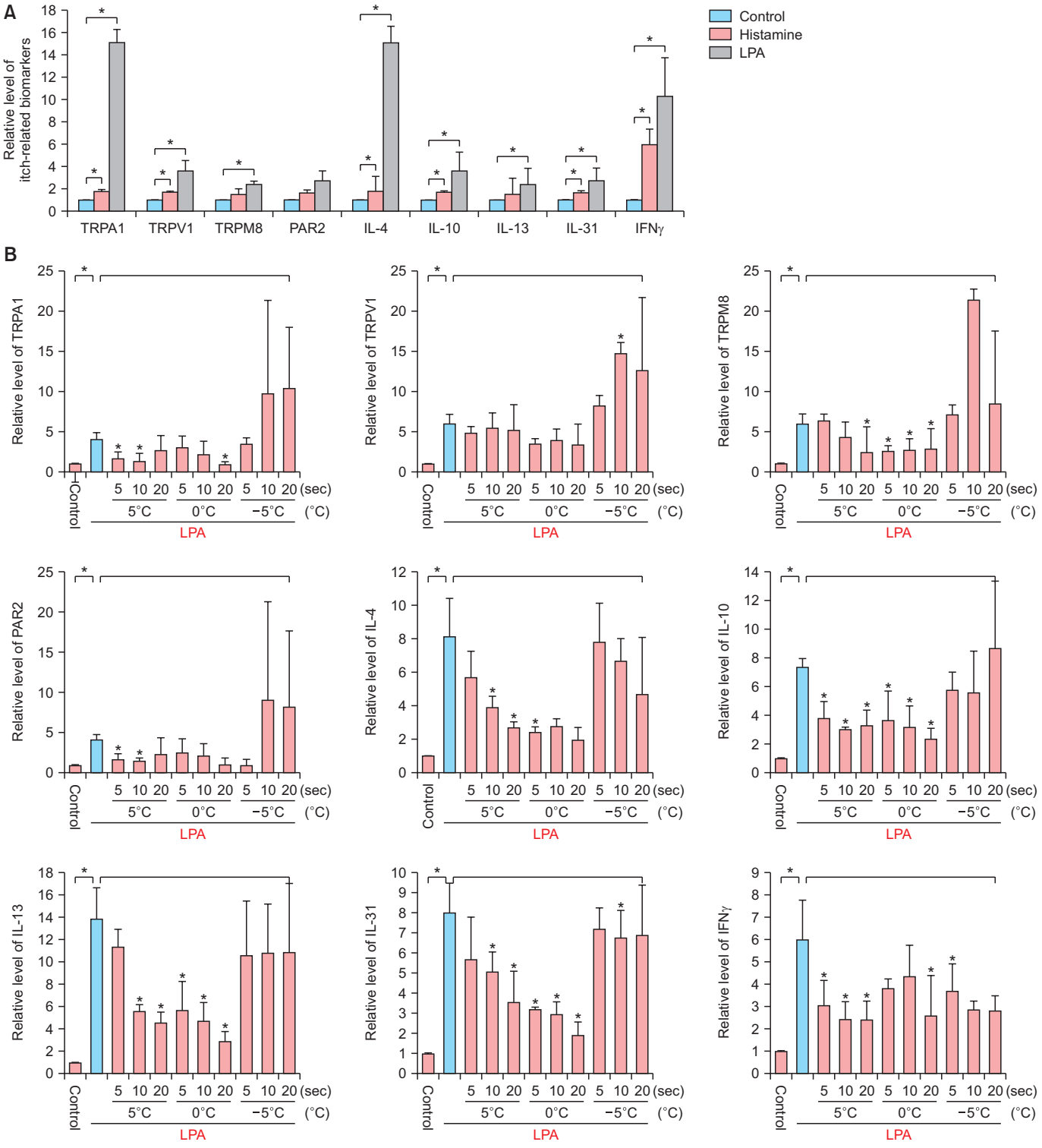

Fig. 2. Continued.

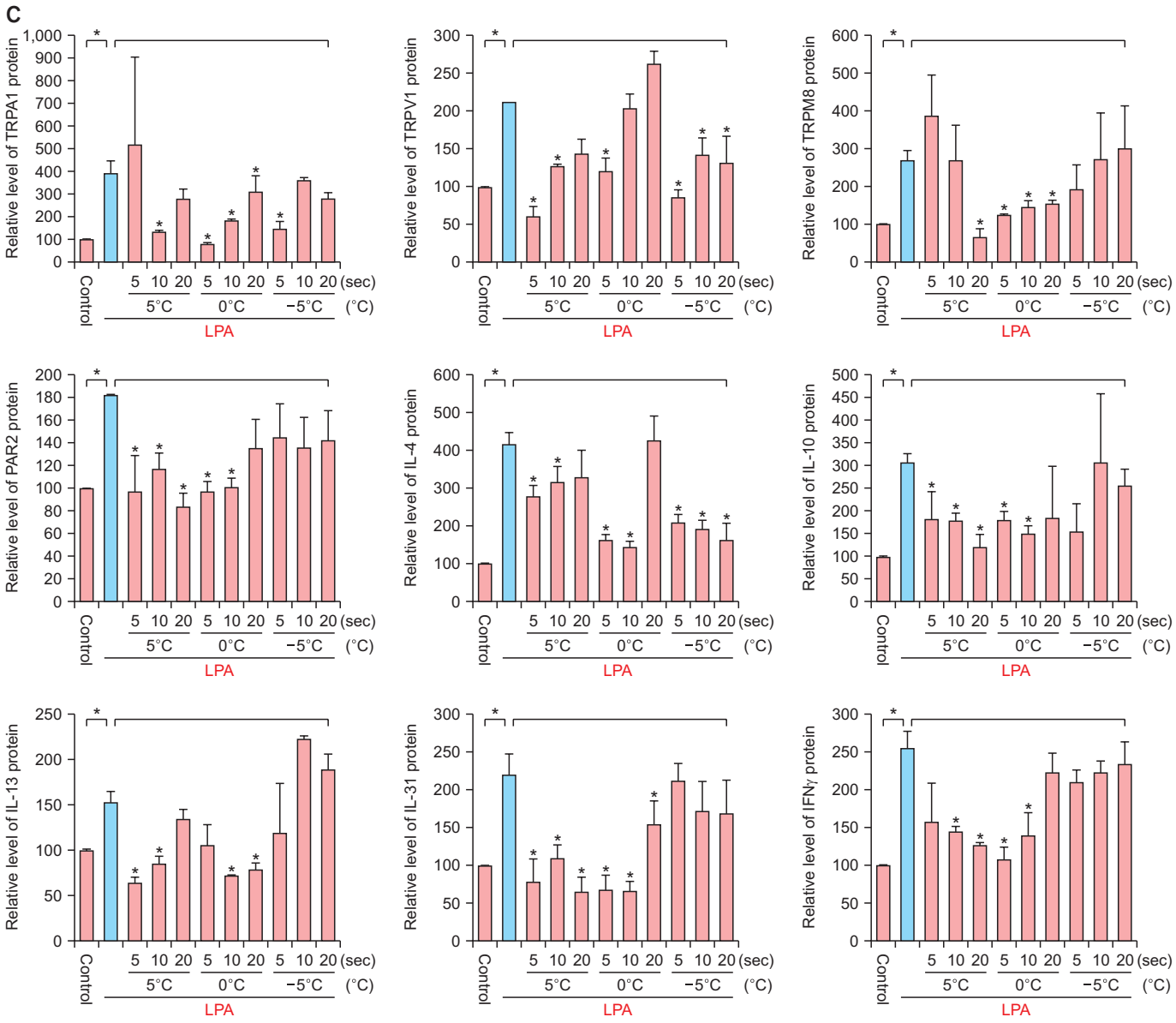

Fig. 2. (A) Gene expression of itch-related biomarkers after treatment with histamine and LPA using real-time PCR. (B) Gene expression of itch-related biomarker decreased after treatment with the cryotherapy device. (C) Protein expression of itch-related biomarkers decreased after treatment with the cryotherapy device. Values are presented as mean±standard deviation. LPA: lysophosphatidic acid, TRPA1: transient receptor potential ankyrin 1, TRPV1: transient receptor potential vanilloid 1, TRPM8: transient receptor potential melastatin 8, PAR2: protease activated receptor 2, IL: interleukin, IFN: interferon. *Statistically significant ($p<0.05$).

be changed with LPA and cryotherapy[10]. We tried cryotherapy at various temperature and time to figure out the most effective therapeutic option for itch because of lack in –20℃ to 10℃ cryotherapy data. As a result, we found out the most effective therapeutic option. Cryotherapy using these options can be developed as an alternative therapeutic tool for chronic pruritus.

SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-21-195-s001.pdf.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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ORCID

Mi Hee Kwack, https://orcid.org/0000-0002-7381-6532
Chang Hyun Song, https://orcid.org/0000-0002-3174-6986
Seongjin Lee, https://orcid.org/0000-0002-2931-392X
Gi Ung Ha, https://orcid.org/0000-0001-6210-7039
Gun-Ho Kim, https://orcid.org/0000-0002-6020-2590
Weon Ju Lee, https://orcid.org/0000-0001-5708-1305

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