Effect of a Precision Cryotherapy Device With Temperature- Adjustability on Mice With Lysophosphatidic Acid-Induced Pruritus

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Research Article

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Abstract

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease that is known to cause a significant adverse impact on the quality of life of patients. Reducing chronic itch that has a complex mechanism is one of the most important challenges in AD treatment.

Objective: To evaluate the effect of a temperature-adjustable cryotherapy device on mice with lysophosphatidic acid-induced pruritus.

Methods: A temperature and time-adjustable cryotherapy device was used for the treatment of lysophosphatidic acid-induced pruritus of mice in the following conditions: −5℃, 0℃, or 5℃ for 5 sec, 10 sec, or 20 sec. Expression of itch-related biomarkers before and after modulation of temperature was investigated with real-time polymerase chain reaction (PCR) and immunohistochemistry.

Results: Expression of itch-related biomarkers was decreased after modulation of temperature. For gene expression, all were decreased at 5℃ for 10 sec and 20 sec, and at 0℃ for 5 sec, 10 sec, and 20 sec. For protein expression, all were decreased at 5℃ for 10 sec and 20 sec, and at 0℃ for 5 sec and 10 sec.

Conclusion: This study demonstrates the pruritus-relieving effect of the temperature-adjustable device on mice. This may provide some evidence for future studies on patients with mild AD.

Introduction

Atopic dermatitis (AD) is a pruritic, relapsing, and chronic inflammatory skin disease that causes a significant adverse impact on the quality of life of patients. In particular, pruritus that persists throughout the daytime and is aggravated at night causes sleep loss and impacts everyday activities as well as the psychosocial health of affected individuals[1,7]. Because chronic itch in AD involves a complex interaction of skin cells, immune cells, secreted factors, and cutaneous neural networks, reduction of this chronic itch is one of the most important challenges in the treatment of AD[13]. Various mediators associated with the nervous system, such as transient receptor potential (TRP) ion channels, are implicated in chronic itch mechanism, in addition to the histaminergic pathways[11,17]. Subtypes of the TRP ion channel superfamily are expressed by sensory nerves, keratinocytes, and certain leukocytes[15]. Particularly, several TRP ion channels play a regulatory role with respect to skin thermal changes that are reported to modulate itch sensitivity[14]. For example, the transient receptor potential melastatin 8 (TRPM8) and the TRPM8-expressing sensory neurons are associated with the antipruritic effects of mild cooling at 20°C[9]. Lysophosphatidic acid (LPA) is a member of the lysopholipid family of bioactive lipids and is an itch mediator that is activated by the transient receptor potential ankyrin 1 (TRPA1) and the transient receptor potential vanilloid 1 (TRPV1)[8].

This study aimed to evaluate the effect of a temperature-adjustable device for cryotherapy on mice with LPA-induced pruritus.

Materials And Methods

Preparation of a device for cryotherapy

A novel cryotherapy system, which can precisely cool a target area from − 20℃ to 10℃, was developed and provided by RecensMedical Inc (Fig. 1a, 1b). This cryotherapy system has a unique capability of regulating the thermodynamic state (temperature, pressure) of cryogenic substance (e.g., carbon dioxide) by applying heat to cryogenic substance. A real-time temperature reading by an IR sensor was used to measure the error between the set cooling temperature and the target temperature, which a feedback controller used to calculate the heat required to achieve a desired thermodynamic state of cryogen substance, leading to rapid and precision cooling at the target area.

Animal study

LPA (3 μM) was injected intradermally in a 10 μl solution in 2 points on the back of 7-week-old female HR-1 mice (SLC Inc., Japan). One week after the injection, the mice were treated with a cryotherapy device with a temperature of either − 5℃ or 5℃ for 5, 10, and 20 sec. The mice were euthanized after 1 day (Fig. 1c). Real-time polymerase chain reaction (PCR) and immunohistochemistry were conducted to evaluate mRNA and protein expression of itch-related biomarkers. This study was approved by the Institutional Animal Care and Use Committee of KNU (No. 2018 − 0167). All methods were carried out in accordance with relevant guidelines and regulations. The study was also carried out in compliance with the ARRIVE guidelines.

Real-time-PCR

Total RNA was isolated using TRIzol reagent, and cDNA was synthesized from 3 mg of total RNA using a cDNA synthesis kit containing the ImProm-II™ reverse transcriptase and oligo-dT primers according to the manufacturer's instructions (Promega, Madison, WI, USA). Real-time PCR was conducted in duplicate with Power SYBR Green premix (Applied Biosystems, Foster City, CA, USA) using 50 ng of cDNA and 10 pM of 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. PCR primer sequences are summarized in Supplementary Table 1. Cycling conditions for amplification were as follows: 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 60 s. The products of PCR were evaluated with a real-time PCR analysis software, the Step one Plus (Applied Biosystems).

Immunohistochemistry

Tissue samples were obtained from the mice and were subsequently placed in cryomolds with embedding medium. Samples were frozen at − 80℃, sliced (7 μm thick), and fixed with 4% paraformaldehyde and 0.1% Triton X-100 for 10 min. After 1 hr preparation with 5% normal donkey serum (Jackson ImmunoResearch), they were incubated overnight at 4℃ with antibodies for TRPA1 (1:100 dilution; Abcam), TRPV1 (1:1000 dilution; Abcam), TRPM8 (1:100 dilution; Abcam), PAR2 (1:100 dilution; Invitrogen), IL-4 (1;400 dilution; Invitrogen), IL-10 (1;200 dilution; Abcam), IL-13 (1;200 dilution; Abcam), IL-

31 (1;200 dilution; Abcam), and IFN-γ (1:200 dilution; Novusbio). The samples were then washed three times with phosphate-buffered saline and incubated with donkey anti-rabbit horseradish peroxidase–conjugated antibody (1:100; Amersham, Buckinghamshire, UK) for 1 hr. 3-Amino9-ethylcarbazole (DAKO, Glostrup, Denmark) was used as a color developing reagent for horseradish peroxidase. The slides were counterstained with hematoxylin for 10 min, followed by counterstaining with DAPI for 10 min. Immunohistochemistry was conducted in duplicate.

Statistical methods

Statistical analysis was done using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA) for Windows. Repeated ANOVA was performed for the data. The level of significance was established at 0.05.

Results

Gene expression of itch-related biomarkers decreased after treatment with the cryotherapy device

Gene expression of itch-related biomarkers was observed to decrease after treatment as follows (Fig. 2): TRPA1 after treatment with 5℃ for 5, 10, and 20 sec, 0℃ for 5, 10, and 20 sec, and − 5℃ for 5 sec; TRPV1 with 5℃ for 5, 10, and 20 sec, 0℃ for 5, 10, and 20 sec; TRPM8 with 5℃ for 10 and 20 sec, 0℃ for 5, 10, and 20 sec; PAR2 with 5℃ for 5, 10, and 20 sec, 0℃ for 5, 10, and 20 sec, and − 5℃ for 5 sec; IL-4, IL-10, IL-13, IL-31, and IFN-γ with 5℃ for 5, 10, and 20 sec, 0℃ for 5, 10, and 20 sec, and − 5℃ for 5, 10, and 20 sec except IL-10 at − 5℃ for 20 sec.

Protein expression of itch-related biomarkers decreased after treatment with the cryotherapy device

Protein expression of itch-related biomarkers was observed to decrease after treatment as follows (Fig. 3): TRPA1 after treatment with 5℃ for 10 and 20 sec, 0℃ for 5, 10, and 20 sec, and − 5℃ for 5, 10, and 20 sec; TRPV1 with 5℃ for 5 and 10 sec, 0℃ for 5, 10, and 20 sec; TRPM8 with 5℃ for 20 sec, 0℃ for 5, 10, and 20 sec, and − 5℃ for 5 sec; PAR2 with 5℃ for 5, 10, and 20 sec, 0℃ for 5, 10, and 20, and − 5℃ for 5, 10, and 20 sec; IL-4, IL-10, IL-13, IL-31, and IFN-γ with 5℃ for 5, 10, and 20 sec, 0℃ for 5, 10, and 20 sec, and − 5℃ for 5, 10, and 20 sec except IL-4 at 0℃ for 20 sec, IL-10 at − 5℃ for 10 sec, and IL-13 at − 5℃ for 10 and 20 sec.

Discussion

Chronic itch that induces scratching is a defining symptom for AD[6]. Pro-inflammatory cytokines from T cells and keratinocytes are considered as a key factor in the pathogenesis of AD and atopic itch[11]. Immunosuppressants and corticosteroids are known to reduce inflammatory components of AD and the resulting itch[5]. These treatment modalities are not effective for every patient with AD because they fail to

target the substantial neural component of the pathophysiology of the itch. Therefore, there is a need for alternative treatments that can directly target neural pathways or intersection between nerves, immune cells, and keratinocytes[17].

Localized skin warming and cooling therapies are believed to be alternative therapeutic modalities that can help relieve the itchy sensation in patients with AD. Additionally, it is known that various itch signaling pathways are differentially modulated by changes in skin temperature [14]. Changes in skin temperature have a marked influence on the intensity of pruritus[5]. In particular, skin cooling is an effective temporary remedy that relieves pruritus in various itchy dermatologic disorders including AD[9]. Cooling can reduce nerve excitability and conduction velocity as well as slow the biochemical mechanisms essential for neurotransmission and neuropeptide release, such as those in itch-mediating C-fibers[2]. In addition, cutaneous vasoconstriction caused by cooling can reduce the release of pruritus-inducing substances[3].

Previous studies have reported that TRPA1, TRPV1, TRPM8, and PAR2 are all involved in the pathogenesis of the itch mechanism[11,16]. The TRP superfamily is known as a major component of the mechanisms of various sensory perceptions including that of itch[10]. Six channels are known to play a regulatory role regarding the thermal/mechanical/chemical transmission in the skin. TRPV-1, -2, -3, and –4 are referred to as “heat channels,” while TRPM8 and TRPA1 are referred to as “cold channels”[15]. Among them, TRPV1, TRPA1, and TRPM8 are expressed in human skin and have been associated with itch. Sander et al.[14] reported that cooling the skin significantly increased serotonin-evoked scratching but reduced histamine-evoked scratching. The increase in serotonin-evoked scratching, but not the reduction of histamine-evoked scratching, was blocked by TRPM8 antagonism.

Interleukin (IL)-2, IL-4, IL-13, and IL-31 used in this experiment are various cytokines and chemokines that have been reported to be correlated with chronic pruritus[11]. In particular, IL-31 is a T helper (Th) 2 cell-derived cytokine that appears to play a critical role in pruritus in AD and is emerging as a new therapeutic target[12]. Systemic and local administration of IL-31 induces itching and scratching in mammals, including humans, probably in a dose-dependent manner[4].

This study was performed to evaluate the possible antipruritic effects of different cooling stimulations at temperatures from − 5°C to 5°C by comparing the expression of gene and protein levels associated with itching and inflammation in a mouse model. In general, it showed that cold temperatures downregulated the expression of itch-related biomarkers at the gene and protein level. According to the temperature and duration of treatments used in the study, the degree of statically significant decrease was different for each biomarker.

In conclusion, these studies have shown that cryotherapy is effective for decreasing pruritus in the AD mouse model, particularly at certain temperatures and durations, resulting in the reduction of itch-related biomarkers.

Furthermore, this study demonstrates the pruritus-relieving effect of the temperature-adjustable device for mice. This may provide some evidence for future studies on patients with mild AD, which may become an opportunity to consider its use as an alternative treatment for chronic itch.

Declarations

Acknowledgment

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Author contributions

W.J.: design, analysis, and manuscript writing. G.H.: analysis and manuscript writing. M.H. and V.M.: design, data collection, manuscript writing. S., H.J., and G.U.: design and manuscript writing. All authors reviewed the manuscript.

Competing Interest

The authors declare no competing interests.

Additional Information

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Data availability

The datasets obtained in the current study are available from the corresponding author on reasonable request.

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Figures

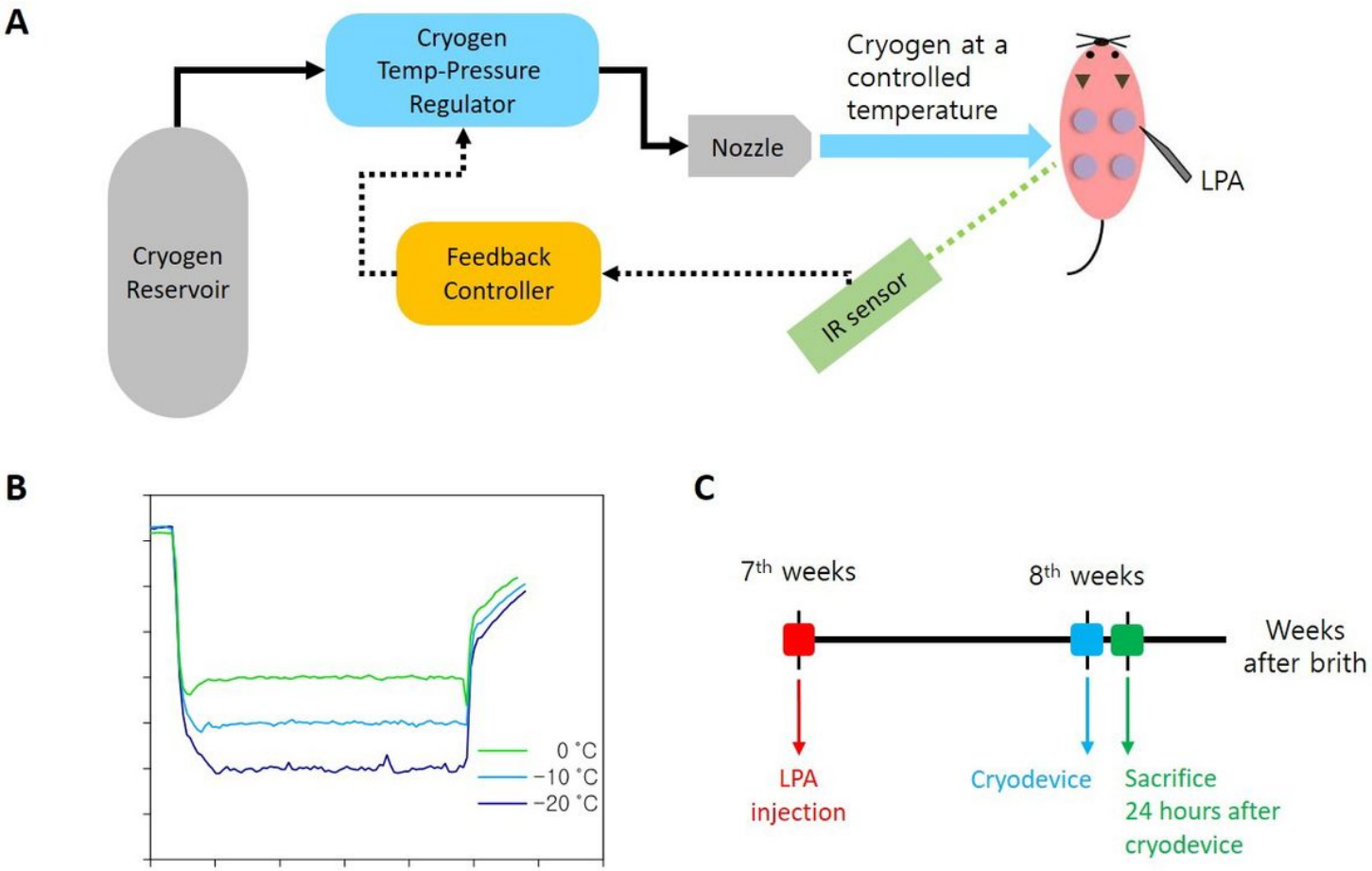

Figure 1

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 control at the target area by the cryotherapy system. (C) Experimental protocol.

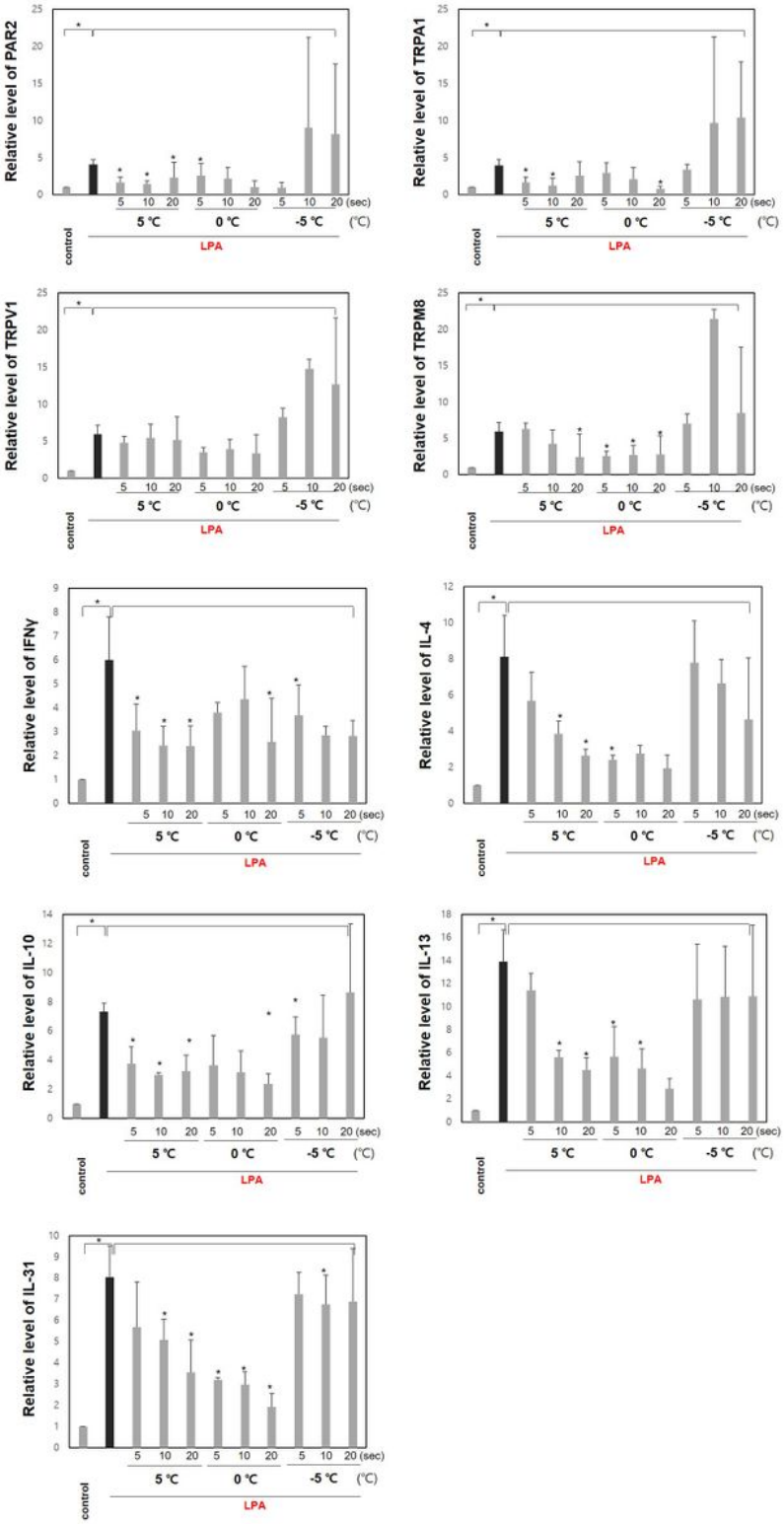

Figure 2

Gene expression of itch-related biomarkers was observed to decrease after treatment with the cryotherapy device. Data are presented as mean ± standard deviation ($p < 0.05$)

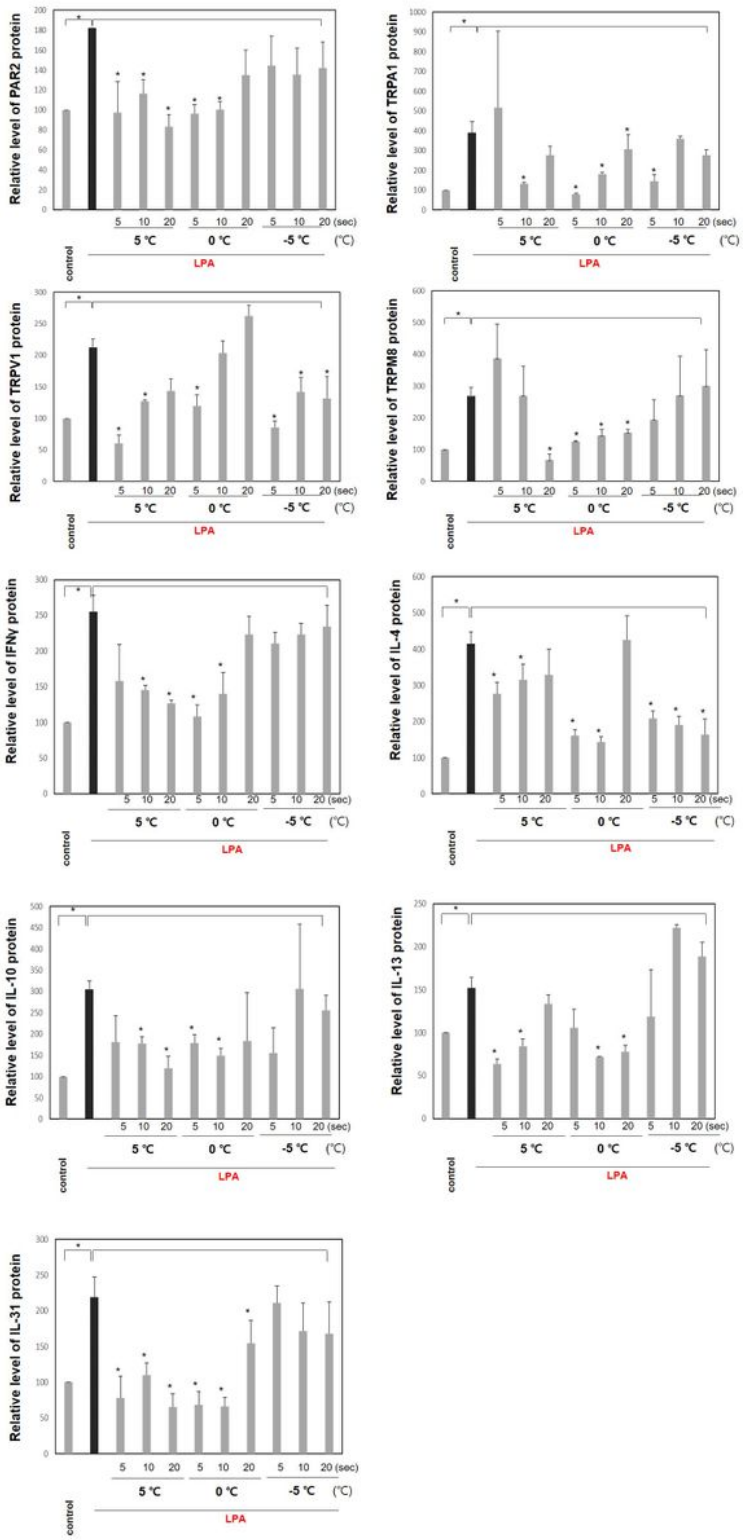

Figure 3

Protein expression of itch-related biomarkers was observed to decrease after treatment with the cryotherapy device. Data are presented as mean ± standard deviation ($p < 0.05$)

Supplementary Files

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- TableSupplementary1.pdf