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Effect of a Precision Cryotherapy Device with Temperature-Adjustability on Mice with *Cutibacterium acnes*-Induced Inflammation

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Dear Editor:

In approximately 80% of young adults and adolescents, acne is a common skin disorder[1]. Acne can be treated with topical, systemic, or surgical therapy, and laser devices and skin care can be added as therapeutic options[2]. However, topical or systemic therapy with retinoids or antibiotics can cause adverse events including skin irritation, dryness, abnormal liver function and bacterial resistance. So, for patients with acne, effective and safer therapeutic modalities are still required. Various physical treatments including cryoslush therapy and cryotherapy are available as acne can be improved by reducing sebum excretion by damaging the sebaceous gland through selective cryolysis[3]. A slush-like mixture consisting of solid carbon dioxide and acetone has been introduced to treat the infected skin[4]. In addition, the cryotherapeutic application of a substance with a very low temperature may lead to good cosmetic results[5]. This study aimed to evaluate the cooling effect on the inflammatory biomarkers' expression.

A novel cryotherapy system, which can precisely cool a target area from –20℃ to 10℃, was developed and provided by RecensMedical Inc. *Cutibacterium (C.) acnes* strain (ATCC 1182) was prepared using storage strain obtained from Korean patients with moderate acne and approval by the Medical Ethical Committee of the Kyungpook National University Hospital for this procedure was waived. *C. acnes* suspensions were prepared as 10^9 colony-forming units/20 μl, and 20 μl aliquots were intradermally injected on 7-week-old female HR-1 mice at four locations on the back (SLC Inc.). Mice were treated with the cryotherapy device at 5℃ (for 5, 10, or 20 sec), 0℃ (for 5, 10, or 20 sec), –5℃ (for 5, 10, or 20 sec), –10℃ (for 5, 10, or 20 sec), or –20℃ (for 5, 10, or 20 sec) 1 week after the injection and euthanized after 1 day. This study was approved by the Institutional Animal Care and Use Committee of KNU (No. 2018-0167). Using the TRIzol reagent, total RNA was isolated, and using a cDNA synthesis kit containing the ImProm-II™ reverse transcriptase and oligo-dT primers, cDNA was synthesized from 3 mg total RNA, according to the manufacturer's instructions (Promega). Real-time PCR was performed in duplicate using the Power SYBR Green premix (Applied Biosystems), 50 ng cDNA, and 10 pM oligonucleotide primers specific for interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-1, MMP-3, and MMP-9. Tissue samples were sliced (7 μm thick), fixed with 4% parafor-

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maldehyde, and treated with 0.1% Triton X-100 for 10 minutes, blocked for 1 hour with 5% normal donkey serum (Jackson ImmunoResearch), and incubated at 4℃ overnight with IL-1β (1:200 dilution; Abcam), IL-6 (1:200 dilution; Abcam), TNF-α (1:200 dilution; R and D), MMP-1 (1:200 dilution; Abcam), MMP-3 (1:200 dilution; Abcam), and MMP-9 (1:250 dilution; Abcam) antibodies. Statistical analysis was done using SPSS version 18.0 (SPSS, Inc.) for Windows. ANOVA was performed for the data. Level of significance was established at 0.05.

The inflammatory nodules' size was measured at eight sites in two mice 1 day after treatment. The *C. acnes*-induced inflammatory nodules' size decreased after treatment at 5℃ (for 5, 10, or 20 sec), 0℃ (for 5, 10, or 20 sec), –5℃ (for 5, 10, or 20 sec), –10℃ (for 5, 10, or 20 sec), or –20℃ (for 5 sec) (Fig. 1). The best cryotherapy option to decrease the inflammatory nodules was 5℃ for 10 seconds. However, there was no significant difference among the cryotherapy options. Gene expression was evaluated in triplicate at the best cryotherapy option. We observed a significant decrease in gene expression of IL-1α, IL-1β, IL-6, TNF-α, MMP-1, MMP-3, and MMP-9 after the cryotherapy device treatment at 5℃ for 10 seconds ($p<0.05$) (Fig. 2A). Immunohistochemical analysis was performed in triplicate at the best cryotherapy option. The expression of IL-1β, IL-6, IL-8, TNF-α, MMP-1, MMP-3, and MMP-9 decreased significantly after cryotherapy with the 5℃ for 10 seconds ($p<0.05$) (Fig. 2B).

Although the traditionally used cryotherapy equipment and methods are simple and relatively inexpensive, cryotherapy tends to be underused. However, cryotherapy is expected to be-

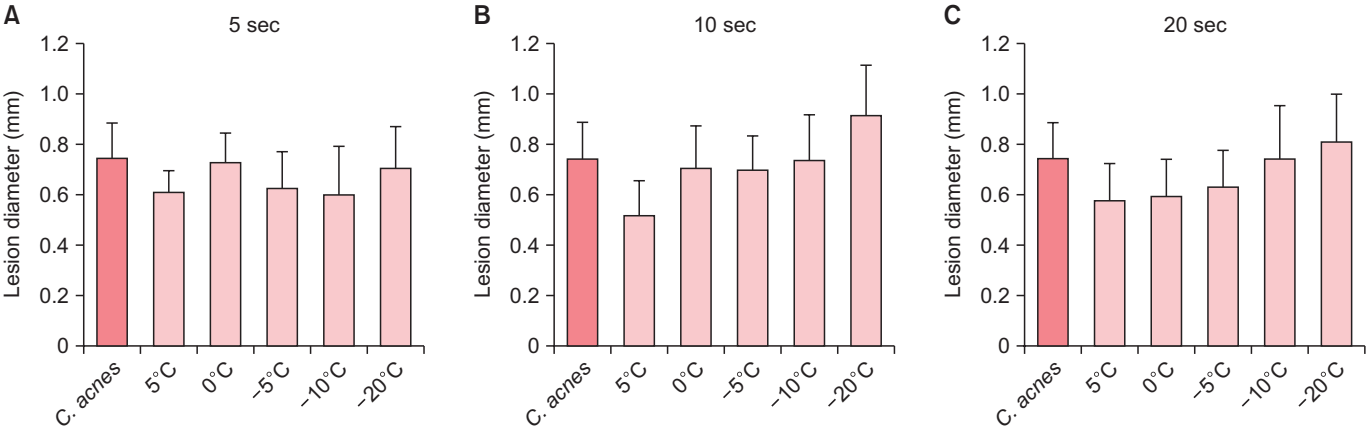

Fig. 1. The inflammatory nodules' size induced by *Cutibacterium acnes* was measured at eight sites in two mice, after treatment for 5 seconds (A), 10 seconds (B), and 20 seconds (C) with the cryotherapy device. The best cryotherapy option to decrease the inflammatory nodules was 5℃ for 10 seconds.

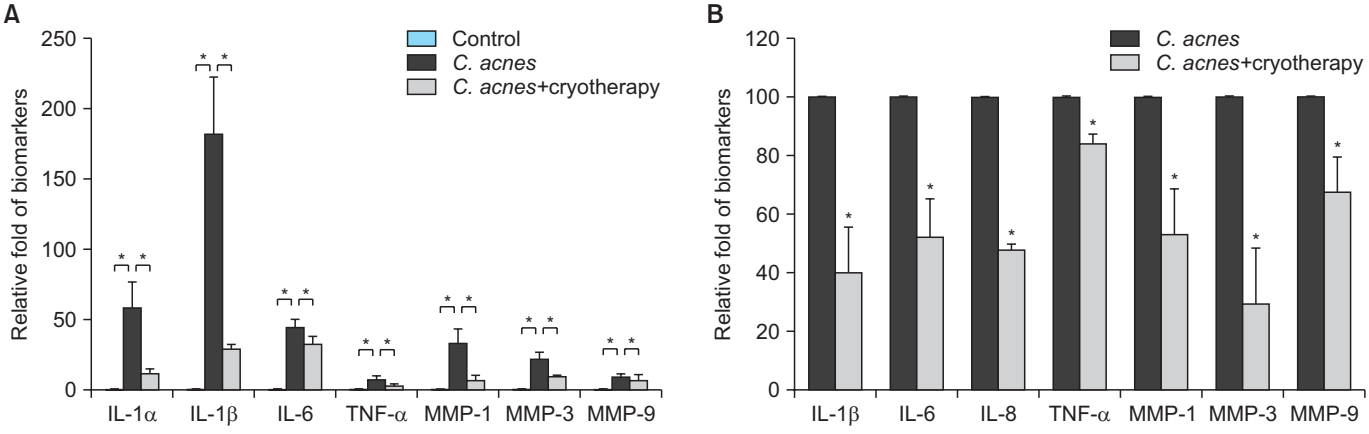

Fig. 2. (A) The cryotherapy device treatment at 5℃ for 10 seconds decreased gene expression of the inflammatory biomarkers, including interleukin (IL)-1α, IL-1β, IL-6, tumor necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-1, MMP-3, and MMP-9. (B) The cryotherapy device treatment at 5℃ for 10 seconds decreased protein expression of the inflammatory biomarkers, including IL-1β, IL-6, IL-8, TNF-α, MMP-1, MMP-3, and MMP-9. *Statistically significant ($p<0.05$).

come an important acne therapeutic option. Cryotherapy applies cryogens, such as liquid nitrogen (–196℃), carbon dioxide (–79℃), nitrous oxide (–90℃), or fluorocarbon liquids (–60℃), on diseased skin[6]. Of these, the most commonly used cryogen in dermatology is liquid nitrogen. Cryogens can be applied with a cryoprobe or using the dipstick method or spot freeze technique[7]. Type, dose, and delivery technique used depend on the lesion to be treated. Solid carbon dioxide slush can be used to treat acne as a peeling agent to reduce the skin's oiliness. Liquid nitrogen on cotton applicators can be used for small and shallow pits as well as for larger acne cysts[8]. The open-spray technique using the paintbrush method has been used to diffuse acne scarring as an alternative to dermabrasion[9]. Cryotherapy has been used as an acne keloidalis therapeutic option because of inflammation reduction. Whole body cryotherapy maintained at –110℃ to –140℃ for 2 minutes increased anti-inflammatory cytokine IL-10 and decreased proinflammatory cytokine IL-2 and chemokine IL-8 as reported by Banfi et al.[10]

In this study, a temperature and time-adjustable cryotherapy device was used for treating *C. acnes*-induced inflammatory nodules on mice. This cryotherapy device can adjust temperatures from –20℃ to 10℃, uses the cryogen carbon dioxide, and utilizes the open-spray, timed spot freeze technique. The inflammatory nodules' size was evaluated after treatment at 5℃ (for 5, 10, or 20 sec), 0℃ (for 5, 10, or 20 sec), –5℃ (for 5, 10, or 20 sec), –10℃ (for 5, 10, or 20 sec), or –20℃ (for 5, 10, or 20 sec). The option of 5℃ for 10 seconds was best for a decrease in inflammatory nodules. In addition, acne-related inflammatory biomarkers' expression was evaluated after treatment at 5℃ for 10 seconds. Cryotherapy using this temperature-adjustable device at 5℃ for 10 seconds decreased the acne-related inflammatory biomarkers' expression. It is believed that this temperature-adjustable cryotherapy device will be a useful therapeutic modality for acne. There was no adverse events in mice after the cryotherapy, including bullae and crusts. However, since mice were euthanized 1 day after cryotherapy, we could not have a chance to observe other adverse events after the cryotherapy, including hypopigmentation and hyperpigmentation. Further studies on how the pigmentation state of the treated area changes over time is needed to determine whether this parameter can be safely used.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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