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Inhibitory effect of cucurbitacin B on imiquimod-induced skin inflammation

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ABSTRACT

Psoriasis is a common skin disease, of which pathogenesis involves the increase of inflammatory reaction in epidermal cells. In an attempt to find therapeutics for psoriasis, we found that cucurbitacin B has an inhibitory potential on imiquimod-induced inflammation of keratinocytes. Cucurbitacin B significantly inhibited imiquimod-induced expression of crucial psoriatic cytokines, such as IL-8 and CCL20, via down-regulation of NF-κB and STAT3 signaling pathway in human keratinocytes. In addition, keratinocyte proliferation was markedly inhibited by cucurbitacin B. The potential beneficial effect of cucurbitacin B on psoriasis was further validated in imiquimod-induced psoriasiform dermatitis of experimental animal. Topical application of cucurbitacin B resulted in significant reduction of epidermal hyperplasia and inflammatory cytokines production, and ameliorated the psoriatic symptom. Taken together, these results suggest that cucurbitacin B may be a potential candidate for the treatment of psoriasis.

1. Introduction

Psoriasis is a common chronic inflammatory skin disease, of which incidence is 0.5–3% of the population worldwide. The characteristic features of psoriasis include keratinocyte hyperproliferation, altered keratinocyte differentiation, and inflammation [1]. For the past three decades, psoriasis has been regarded as an adaptive immune-mediated disease, in which Th1-type immune cells and their cytokines are critically involved in the pathogenesis [2]. In addition, recent findings add new concept to the pathogenesis of psoriasis, explaining the importance of keratinocytes as the primary defense cells against environmental insults. That is, psoriasis can be triggered and/or exacerbated by physical trauma on the skin, suggesting that keratinocytes may be the potential origin cells for psoriasis with a burst of innate immunity. Keratinocytes interact with immune cells to expand the immune response. Besides, keratinocytes can contribute to immune surveillance through the expression of a range of Toll-like receptors (TLRs), the pattern recognition receptors (PRRs) in human innate immunity [3–5]. Recognition of bacterial pathogen-associated molecular patterns (PAMPs) by keratinocytes results in activation of inflammation-related intracellular signaling such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and production of inflammatory cytokines from keratinocytes. Thus, therapeutics targeting the inflammatory reaction of keratinocytes is one promising approach to develop novel therapeutics for psoriasis.

Imiquimod is an analog of adenosine, which exerts its action as a specific TLR7 agonist [6]. Stimulation of keratinocytes by imiquimod leads to activation of NF-κB signaling and subsequent inflammatory reaction [7,8]. In addition, topical treatment of 5% imiquimod cream induces psoriasiform dermatitis in human and mouse [9,10]. Although there is an evidence that Aldara (5% imiquimod cream) induces inflammation independently of TLR7 [11], imiquimod-induced inflammation model is widely used for psoriasis research. Thus, we attempted to find therapeutics on psoriasis, using this model. We found that cucurbitacin B has an inhibitory

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potential on imiquimod-induced inflammation of keratinocytes. Cucurbitacin B is a phytochemical isolated from cucurbitaceae plants. It has been shown that cucurbitacin B has anti-cancer and anti-inflammatory effects [12]. However, the effect of cucurbitacin B on inflammatory reaction in epidermal keratinocytes remains unclear. In this study, we demonstrate that cucurbitacin B inhibits imiquimod-induced inflammatory reaction in keratinocytes, suggesting that cucurbitacin B may be developed for psoriasis treatment.

2. Materials and methods

2.1. Cell culture

Human skin tissues were obtained under the written informed consent of donors, in accordance with the ethical committee approval process of the Institutional Review Board of Chungnam National University Hospital. Primary keratinocytes were cultured according to the method previously described [13]. For immortalization, keratinocytes were transduced with the recombinant retrovirus expressing simian virus 40 T antigen (SV40Tag) and selected using G418 for 4 weeks [14]. SV40Tag-transformed human epidermal keratinocytes (SV-HEKs) were routinely cultured in keratinocyte-serum free medium (K-SFM) supplemented with bovine pituitary extract (BPE) and recombinant human epidermal growth factor (rhEGF) (Life Technologies Corporation, Grand Island, NY). For treatment of SV-HEKs cultured in vitro, cucurbitacin B was purchased from Sigma–Aldrich (St. Louis, MO) and imiquimod was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Cell viability test

SV-HEKs were seeded in 6-well plate at a density of 2×10^5, treated with cucurbitacin B for 24 h. After treatment, cells received 2 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution and were incubated for a further 4 h. Cell viability was determined by measuring optical density at 540 nm using an ELISA reader.

2.3. Cell growth analysis

SV-HEKs were seeded in 60-mm culture dish, treated with cucurbitacin B in the presence of 1 μCi of [^{3}H]thymidine (PerkinElmer, Boston, MA). Following incubation for the indicated time points, cells were washed twice with PBS and incubated with 0.1 N NaOH. Radioactivity in cell lysates was measured by liquid scintillation counter.

2.4. Quantitative real-time polymerase chain reaction (qPCR)

Total RNAs were isolated using Easy-blue RNA extraction kit (Intron, Daejeon, Korea). Two μg of total RNAs were reverse transcribed with moloney-murine leukaemia virus (M-MLV) reverse transcriptase (RTase) (ELPIS Biotech, Daejeon, Korea). Aliquots of RT

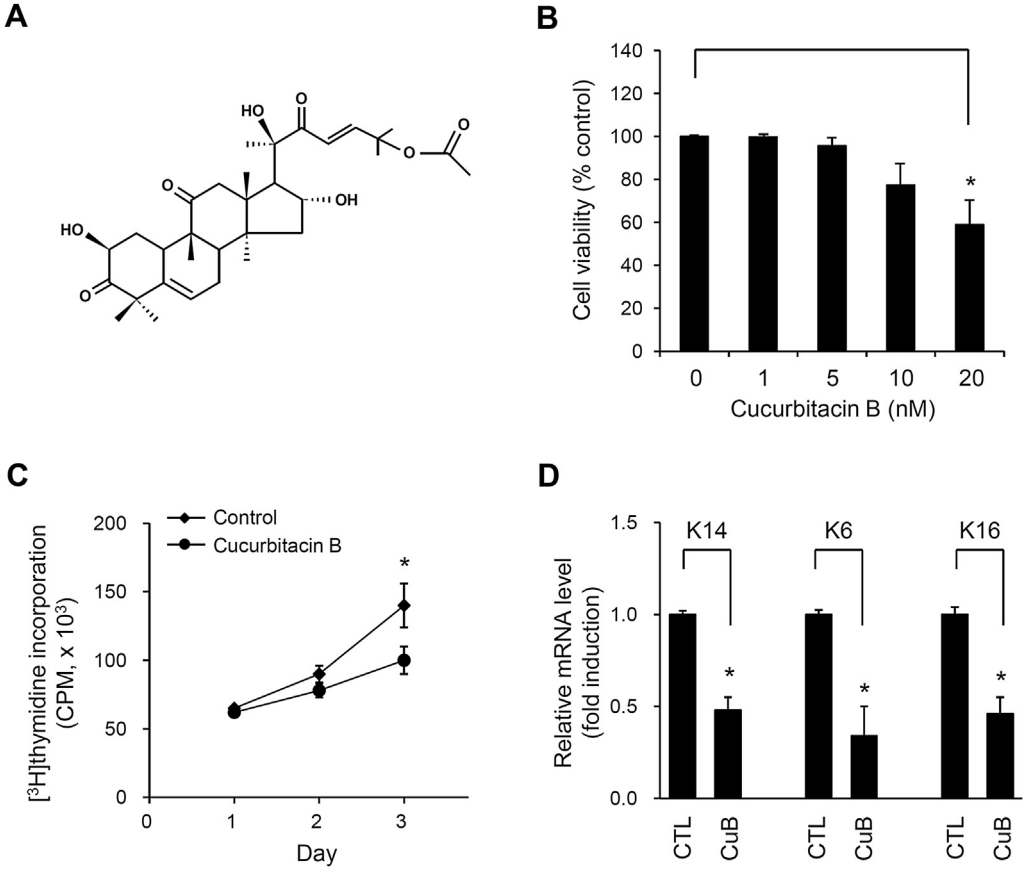

Fig. 1. (A) Structure of cucurbitacin B. (B) Cytotoxicity of cucurbitacin B. SV40Tag-transformed human epidermal keratinocytes (SV-HEKs) were treated with cucurbitacin B at the indicated concentrations for 2 d. Cell viability was measured by MTT assay. (C) Effect of cucurbitacin B on the cell growth. Cells were treated with 5 nM cucurbitacin B for the indicated time points in the presence of 1 μCi of [^{3}H]thymidine. Cells were lysed using 0.1 N NaOH and radioactivity was measured. Data are expressed as count per minute (CPM). (D) Effect of cucurbitacin B on the expression of proliferation-related keratins. Cells were treated with 5 nM cucurbitacin B for 2 d. The mRNA level was determined by qPCR. Data are expressed as fold induction. The mean values ± SD are averages of triplicate measurements. $^*P < 0.01$. K14; keratin 14, K6; keratin 6, K16; keratin 16.

mixture were amplified using SYBR Green real-time PCR master mix (ELPIS Biotech). The following primers sequences were used: human IL-8, 5′-TTTCCACCCCAAATTTATCA and 5′-TTTCTGTGTT GGCGCAGTGT; human CCL20, 5′-CCACCTCTGCGGCGAAT and 5′-CGGTCTGTGTATCCAAGACA; human β-defesin-2, 5′-GCCTCTTCCAG GTGTTTTTG and 5′-CTCCACTCTTAAGGCAGGTA; human β-defesin-3, 5′-GGTGCCTGTTCCAGGTCATG and 5′-CGCCTCTGACTCTGCAA-TAA; human keratin 14, 5′-GGCCTGCTGAGATCAAAGAC and 5′-GTCCACTGTGGCTGTGAGAA; human keratin 6, 5′-TTTGTGACTCT-GAAGAAGGA and 5′-GCCTTGGCTTGCAGTTCAAC; human keratin16, 5′-TGCTGGAGGGCGAGGAT and 5′-ATAGGATTGGCCAGATGCTT; mouse keratin 16, 5′-GCGGCCCACTGAGATCAA and 5′-CTTGCTCTTC AGGTCCTCAA; mouse IL-8, 5′-GCTCCTGCTGGCTGTCCTTA and 5′-CGTAGCTCTTGAGTGTCACA.

2.5. ELISA

Culture medium was collected, and secreted CCL20 and IL-8 were determined using commercial ELISA kits. CCL20 kit was purchased from MyBioSource (San Diego, CA), and IL-8 kit was purchased from Life Technologies Corporation (Grand Island, NY).

2.6. Luciferase reporter assay

SV-HEKs were seeded in 6-well dishes, then transduced with 1 multiplicity of infection (MOI) of NF-κB reporter adenovirus for 6 h. After replenishing with fresh medium, cells were treated with cucurbitacin B for 24 h. In some group, cells were also treated with imiquimod 1 h after addition of cucurbitacin B. Cells were harvested and luciferase activity was measured using the dual luciferase reporter assay system (Promega, Madison, WI).

2.7. Western blotting

Cells were lysed in Proprep solution (Intron, Daejeon, Korea). Total protein was measured using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). Samples were run on SDS-polyacrylamide gels, transferred to nitrocellulose membranes and incubated with appropriate antibodies. Blots were then incubated with peroxidase-conjugated secondary antibodies, visualized by enhanced chemiluminescence (Intron). The following primary antibodies were used in this study: phospho-p65, phospho-IκBα, IκBα, phospho-STAT3, and STAT3 (Cell Signaling Technology, Beverly, MA), actin (Sigma–Aldrich).

2.8. Animal test

Male BALB/c mice at 6–8 weeks of age were purchased from Orient Bio (Seongnam, Korea). The psoriasiform skin inflammation was generated by topical application of 5% imiquimod cream (Aldara; 3M Health Care Ltd., Leicestershire, UK) daily for 7 d [15]. Cucurbitacin B was dissolved in DMSO and diluted with acetone to the desired concentrations, and pretreated 1 h before imiquimod application.

2.9. Immunohistochemistry

Paraffin sections were incubated with H_2O_2 for 10 min to block endogenous peroxidase and then soaked in 5% bovine serum albumin for 30 min. Sections were incubated with appropriate primary antibodies. Sections were incubated sequentially with peroxidase-conjugated secondary antibody and visualized with Chemmate envision detection kit (Dako, Carpinteria, CA).

2.10. Statistical analysis

Data were evaluated statistically using one-way analysis of variance (ANOVA) with the SPSS software (v 22.0; IBM, Seoul, Korea). Statistical significance was set at $p < 0.01$.

3. Results

In an attempt to find potential therapeutics for psoriasis, we found that cucurbitacin B may be a good candidate for that purpose. Cucurbitacin B is a phytochemical isolated from cucurbitaceae plants, and classified as a steroid molecule (Fig. 1A). We first determined the cytotoxicity of cucurbitacin B on keratinocytes. When SV-HEKs were treated with cucurbitacin B, it did not show the cytotoxicity up to the dose of 5 nM (Fig. 1B). As psoriasis is a disease related to the hyper-proliferation of keratinocytes, we next tested whether cucurbitacin B affects cell growth. When SV-HEKs were treated with 5 nM cucurbitacin B, cell growth was significantly retarded (Fig. 1C). Concomitantly, cucurbitacin B treatment resulted in down-regulation of proliferation-related keratins such as keratin 14, 6 and 16 (Fig. 1D).

Imiquimod is an immune response modifier that is widely used for the treatment of skin cancers such as basal cell carcinoma, Bowen's disease, and actinic keratosis [16]. However, imiquimod induces psoriasiform dermatitis in human and mouse, and it also induces production of inflammatory cytokines from keratinocytes [10,17]. We examined whether cucurbitacin B affects imiquimod-induced immune response in keratinocytes. Imiquimod increased the release of inflammatory cytokines CCL20 and IL-8 from SV-HEKs, while cucurbitacin B pretreatment significantly inhibited the imiquimod-induced cytokine release (Fig. 2A). Consistent with

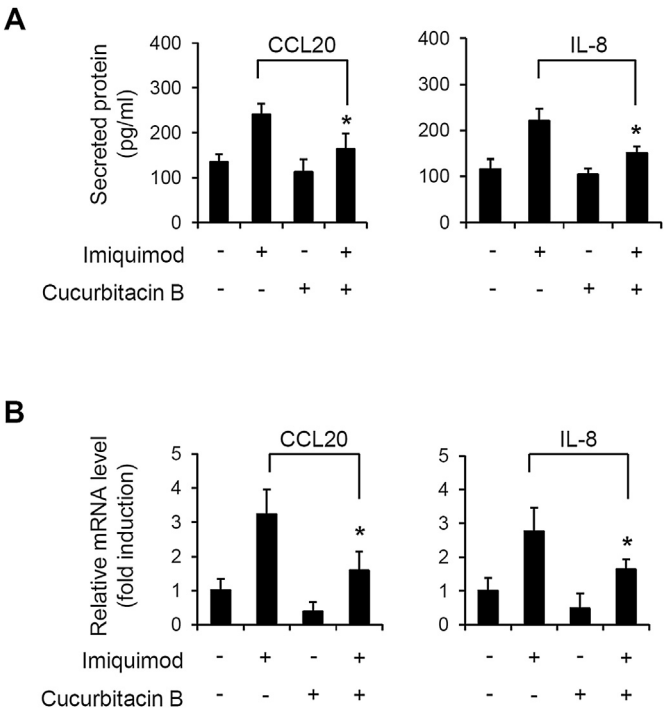

Fig. 2. Effect of cucurbitacin B on imiquimod-induced inflammatory reaction in keratinocytes. (A) SV-HKEs were pretreated with 5 nM cucurbitacin B for 1 h, and then stimulated with 5 μg/ml imiquimod for 24 h. Released cytokines were measured by ELISA. (B) SV-HKEs were pretreated with 5 nM cucurbitacin B for 1 h, and then stimulated with 5 μg/ml imiquimod for 6 h. The mRNA level was determined by qPCR. Data are expressed as fold induction. The mean values ± SD are averages of triplicate measurements. *$P < 0.01$.

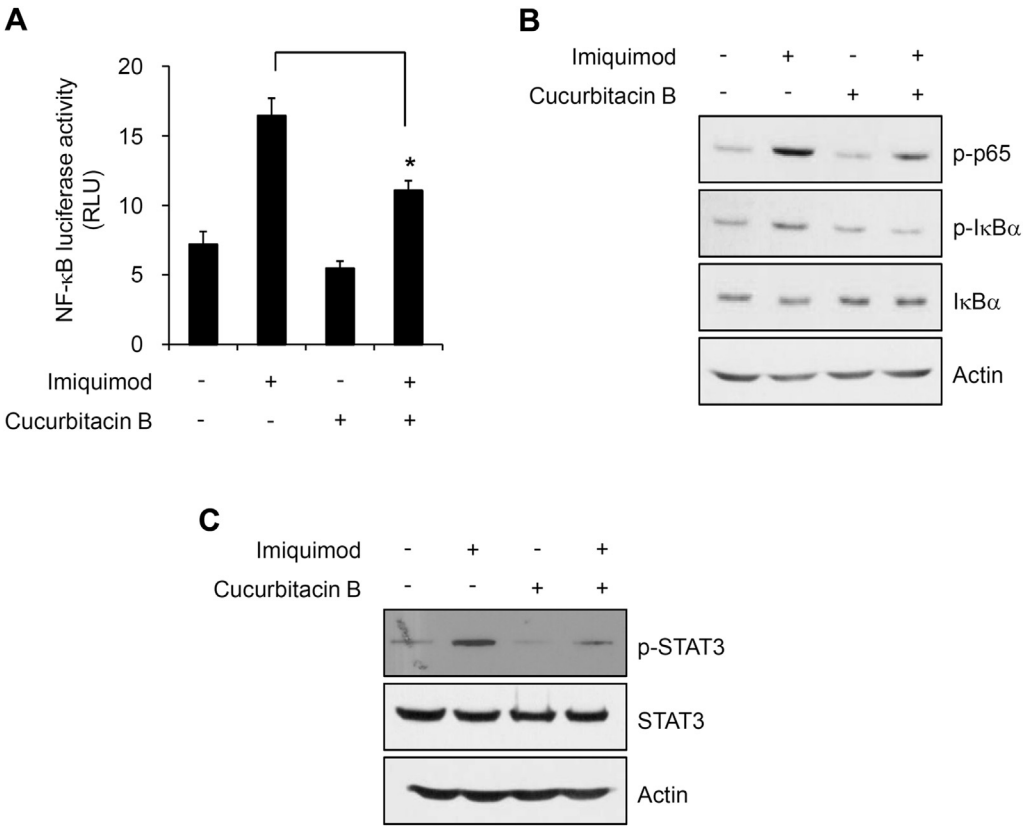

Fig. 3. Effect of cucurbitacin B on imiquimod-induced intracellular signaling in keratinocytes. (A) SV-HEKs were transduced with adenovirus harboring NF-κB-luciferase reporter, then treated with imiquimod (5 μg/ml) and cucurbitacin B (5 nM). Cells were lysed and assayed for luciferase activity. Data are represented as relative light unit (RLU). The mean values ± SD are averages of triplicate measurements. $^{*}P < 0.01$. (B) Activation of NF-κB signaling was determined by Western blot. The protein level for phoshorylated-p65 (p-p65) and phosphorylated-IκBα (p-IκBα) was decreased by cucurbitacin B pretreatment. (C) Cucurbitacin B inhibited imiquimod-induced phosphorylation of STAT3 (p-STAT3).

these results, imiquimod increased mRNA levels for CCL20 and IL-8, and cucurbitacin B pretreatment significantly inhibited cytokine gene expression (Fig. 2B).

To investigate putative action mechanism, we examined the effect of cucurbitacin B on NF-κB signaling, the central player in inflammatory reaction. Imiquimod increased NF-κB activity, which was significantly inhibited by cucurbitacin B (Fig. 3A). Consistent with this result, Western blot showed that imiquimod-induced phosphorylation of p65 and IκBα was markedly inhibited by cucurbitacin B (Fig. 3B). As signal transducer and activator of transcription 3 (STAT3) is another important player linked to psoriasis [18], we checked the effect of cucurbitacin B on this signaling pathway. Similar to the results on NF-κB signaling, cucurbitacin B markedly inhibited imiquimod-induced phosphorylation of STAT3 (Fig. 3C).

To further evaluate the potential effect of cucurbitacin B, we performed animal test. Consistent with previous reports [15,19], daily topical application of 5% imiquimod cream (Aldara) induced psoriasiform dermatitis, in which epidermal thickness was remarkably increased. When cucurbitacin B was pretreated, induction of psoriasiform dermatitis was significantly inhibited. Histochemistry study clearly showed that cucurbitacin B prevented imiquimod-induced epidermal hyperplasia. And this result was tightly linked to the decrease of proliferating cells in epidermis, evidenced by reduction of Ki67 positive cells in cucurbitacin B-treated group. In addition, imiquimod-induced STAT3 phosphorylation was also markedly inhibited by cucurbitacin B (Fig. 4A). Quantification confirmed that imiquimod-induced epidermal thickening was markedly inhibited by cucurbitacin B (Fig. 4B). Finally, topical application of cucurbitacin B significantly inhibited imiquimod-induced gene expression for keratin 16 and IL-8, the characteristic molecules increased in psoriasis (Fig. 4C). Together, these data suggest that cucurbitacin B can be applicable for the treatment of psoriasis.

4. Discussion

Keratinocytes are the major cells of epidermis, and play a key role to make skin barrier. The primary function of skin barrier is the protection against environmental insults such as microbial infection, noxious chemicals and ultraviolet (UV). Keratinocytes provide physical components of barrier structure, including cornified cell envelope proteins and surrounding lipids [20]. In addition to this essential role for providing building blocks of skin barrier, it has been recognized that keratinocytes function as the primary defense cells against non-self and/or self antigens. Evidence shows that keratinocytes express many TLR molecules and engage in innate immune response [4]. With respect to the pathogenesis of psoriasis, keratinocytes produce a range of inflammatory cytokines, thereby functioning to recruit and activate immune cells such as neutrophils and activated T cells [21]. In this regard, the approach that inhibits inflammatory reaction in keratinocytes is one attractable method for treatment of psoriasis. In this study, we demonstrated that cucurbitacin B inhibited imiquimod-induced inflammatory reaction in cultured keratinocytes. We also demonstrated that cucurbitacin B inhibited imiquimod-induced psoriasiform dermatitis in animal model.

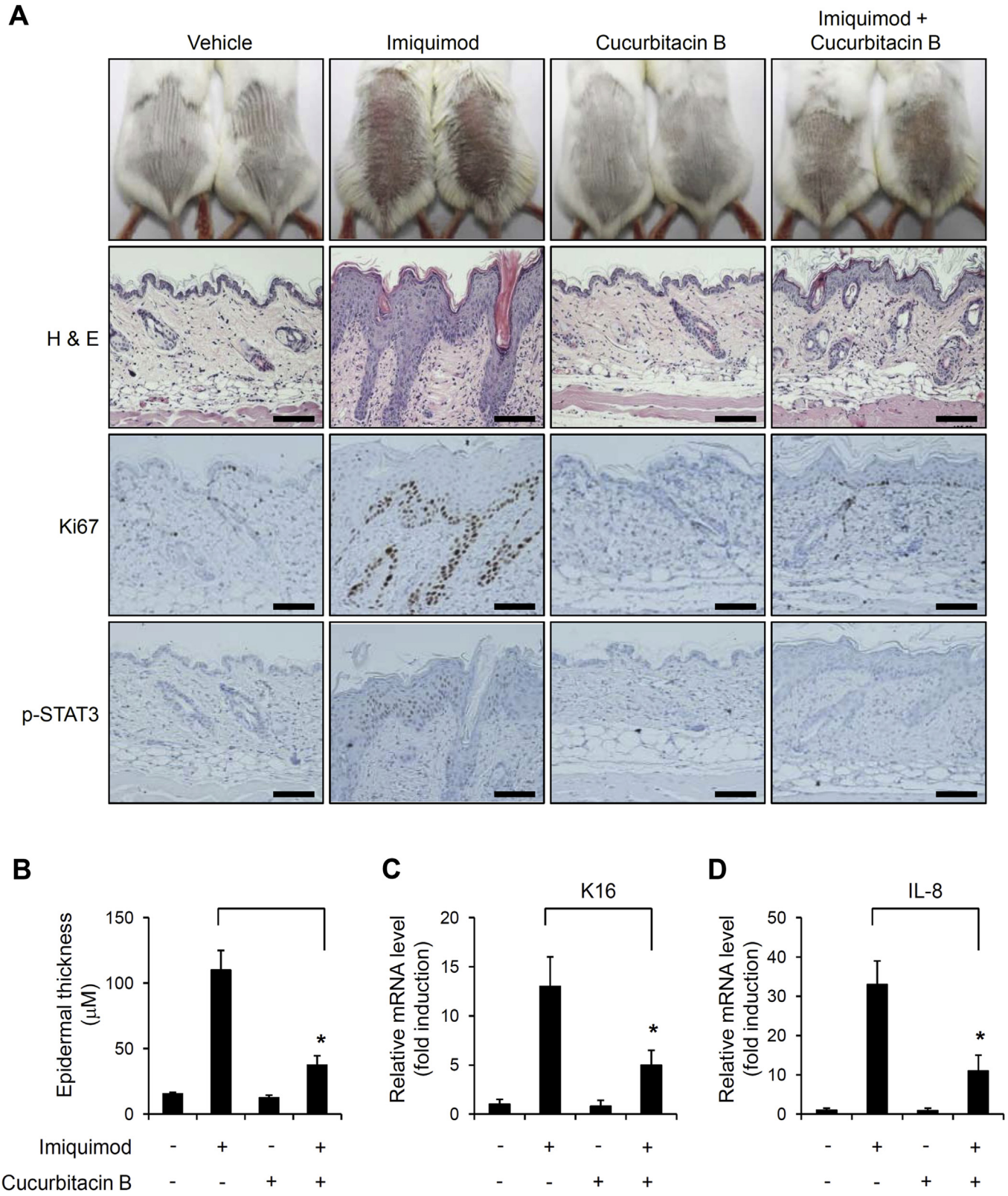

Fig. 4. Inhibition of imiquimod-induced psoriasiform dermatitis by cucurbitacin B. (A) BALB/c mice were topically applied with 5% imiquimod cream (Aldara) for 7 d. Cucurbitacin B (100 nM) was pretreated 1 h before imiquimod application. Macroscopic appearance of the skin was observed at the end of the experiment, and skin specimens were further investigated (H & E; hematoxylin and eosin staining). Immunohistochemistry analysis was performed to determine the cell proliferation in epidermis (Ki67 staining), and activation of STAT signaling (p-STAT3). (B) Epidermal thickness was measured. Data are the mean values ± SD ($n = 5$). $^{*}P < 0.01$. (C) Total RNA was isolated from skin tissue, and then mRNA level for keratin 16 (K16) was determined by qPCR. (D) The mRNA level for IL-8 was also determined. Data are expressed as fold induction. The mean values ± SD are averages of triplicate measurements. $^{*}P < 0.01$.

Cucurbitacins are biochemical compounds isolated from cucurbitaceae plants, and classified as steroids. There are many of cucurbitacin variants, from cucurbitacin A to cucurbitacin T. Multiple pharmacological activities, including neuroprotective, anti-proliferative, and apoptosis-inducing effect, have been reported [22–24]. With respect to cucurbitacin B, several studies have demonstrated that cucurbitacin B has anti-cancer and anti-inflammatory potential. For example, cucurbitacin B inhibits the proliferation of BRCA1-defective breast cancer cells via up-regulation of $p21^{waf}$ and $p27^{Kip1}$ [25]. Other evidence shows that cucurbitacin B inhibits hepatocellular carcinoma cell growth via the inhibition of phosphorylation of STAT3 [26]. Additionally, cucurbitacin B shows inhibitory effect on TPA-induced inflammation in mouse ear [27].

In this study, we demonstrated that cucurbitacin B reduced the growth of keratinocytes cultured in vitro. In a previous study, cucurbitacin B up-regulates the cyclin-dependent kinase (CDK) inhibitors such as $p21^{waf}$ and $p27^{Kip1}$, thereby attenuating cell growth in breast cancer cells. Similarly, there is a possibility that cucurbitacin B inhibits the cell growth via the modulation of cell cycle regulators in keratinocytes. The precise mechanism underlying cell growth inhibition should be investigated further. Nevertheless, the cell growth-inhibiting potential of cucurbitacin B should be emphasized in the context of developing therapeutics, because that hyper-proliferation of keratinocytes is a specific character of psoriasis. It is expected that reducing the keratinocyte growth helps to relieve the psoriatic symptom.

The intracellular signaling molecules such as NF-κB and STAT3 have long been recognized as the key players in the pathogenesis of psoriasis. In this study, we demonstrated that cucurbitacin B markedly inhibited imiquimod-induced activation of NF-κB and STAT3. Thus, it can simply be speculated that anti-psoriatic effect of cucurbitacin B may be due to its action on intracellular signaling cascades. Interestingly, previous study indicates that steroid molecule dexamethasone inhibits the activation of NF-κB and STAT3 in acute pancreatitis model [28]. As cucurbitacin B is classified as a steroid, there is a possibility that cucurbitacin B exerts its action in a similar way to glucocorticoids. Elucidation of precise action mechanism will be an interesting further study.

In summary, we demonstrated that cucurbitacin B has a potential for inhibiting imiquimod-induced inflammatory reaction in keratinocytes. Our data suggest that cucurbitacin B would be a promising candidate for the treatment of psoriasis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Transparency document

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References

[1] M.A. Lowes, A.M. Bowcock, J.G. Krueger, Pathogenesis and therapy of psoriasis, Nature 445 (2007) 866–873.
[2] Y. Li, A.B. Begovich, Unraveling the genetics of complex diseases: susceptibility genes for rheumatoid arthritis and psoriasis, Semin. Immunol. 21 (2009) 318–327.
[3] R. Medzhitov, Toll-like receptors and innate immunity, Nat. Rev. Immunol. 1 (2001) 135–145.
[4] J.E. McInturff, R.L. Modlin, J. Kim, The role of toll-like receptors in the pathogenesis and treatment of dermatological disease, J. Invest. Dermatol 125 (2005) 1–8.
[5] G. Trinchieri, A. Sher, Cooperation of toll-like receptor signals in innate immune defence, Nat. Rev. Immunol. 7 (2007) 179–190.
[6] H. Hemmi, T. Kaisho, O. Takeuchi, S. Sato, H. Sanjo, K. Hoshino, T. Horiuchi, H. Tomizawa, K. Takeda, S. Akira, Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway, Nat. Immunol. 3 (2002) 196–200.
[7] T. Kono, S. Kondo, S. Pastore, G.M. Shivji, M.A. Tomai, R.C. McKenzie, D.N. Sauder, Effects of a novel topical immunomodulator, imiquimod, on keratinocyte cytokine gene expression, Lymphokine Cytokine Res. 13 (1994) 71–76.
[8] M.P. Schön, M. Schön, K.N. Klotz, The small antitumoral immune response modifier imiquimod interacts with adenosine receptor signaling in a TLR7- and TLR8-independent fashion, J. Invest. Dermatol 126 (2006) 1338–1347.
[9] M. Gilliet, C. Conrad, M. Geiges, A. Cozzio, W. Thürlimann, G. Burg, F.O. Nestle, R. Dummer, Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors, Arch. Dermatol 140 (2004) 1490–1495.
[10] B. Flutter, F.O. Nestle, TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis, Eur. J. Immunol. 43 (2013) 3138–3146.
[11] A. Walter, M. Schäfer, V. Cecconi, C. Matter, M. Urosevic-Maiwald, B. Belloni, N. Schönewolf, R. Dummer, W. Bloch, S. Werner, H.D. Beer, A. Knuth, M. van den Broek, Aldara activates TLR7-independent immune defence, Nat. Commun. 4 (2013) 1560.
[12] B. Jayaprakasam, N.P. Seeram, M.G. Nair, Anticancer and antiinflammatory activities of cucurbitacins from Cucurbita andreana, Cancer Lett. 189 (2003) 11–16.
[13] J.S. Lee, D.H. Kim, D.K. Choi, C.D. Kim, G.B. Ahn, T.Y. Yoon, J.H. Lee, J.Y. Lee, Comparison of gene expression profiles between keratinocytes, melanocytes and fibroblasts, Ann. Dermatol 25 (2013) 36–45.
[14] H.I. Choi, K.C. Sohn, D.K. Hong, Y. Lee, C.D. Kim, T.J. Yoon, J.W. Park, S. Jung, J.H. Lee, Y.H. Lee, Melanosome uptake is associated with the proliferation and differentiation of keratinocytes, Arch. Dermatol. Res. 306 (2014) 59–66.
[15] R.M. Andrés, M.C. Montesinos, P. Navalón, M. Payá, M.C. Terencio, NF-κB and STAT3 inhibition as a therapeutic strategy in psoriasis: in vitro and in vivo effects of BTH, J. Invest. Dermatol 133 (2013) 2362–2371.
[16] A.O. Huen, A.H. Rook, Toll receptor agonist therapy of skin cancer and cutaneous T-cell lymphoma, Curr. Opin. Oncol. 26 (2014) 237–244.
[17] Z.J. Li, K.C. Sohn, D.K. Choi, G. Shi, D. Hong, H.E. Lee, K.U. Whang, Y.H. Lee, M. Im, Y. Lee, Y.J. Seo, C.D. Kim, J.H. Lee, Roles of TLR7 in activation of NF-κB signaling of keratinocytes by imiquimod, PLoS One 8 (2013) e77159.
[18] S. Sano, K.S. Chan, S. Carbajal, J. Clifford, M. Peavey, K. Kiguchi, S. Itami, B.J. Nickoloff, J. DiGiovanni, Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model, Nat. Med. 11 (2005) 43–49.
[19] L. van der Fits, S. Mourits, J.S. Voerman, M. Kant, L. Boon, J.D. Laman, F. Cornelissen, A.M. Mus, E. Florencia, E.P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, J. Immunol. 182 (2009) 5836–5845.
[20] A.E. Kalinin, A.V. Kajava, P.M. Steinert, Epithelial barrier function: assembly and structural features of the cornified cell envelope, Bioessays 24 (2002) 789–800.
[21] C.M. Sweeney, A.M. Tobin, B. Kirby, Innate immunity in the pathogenesis of psoriasis, Arch. Dermatol. Res. 303 (2011) 691–705.
[22] A.M. Arel-Dubeau, F. Longpré, J. Bournival, C. Tremblay, J. Demers-Lamarche, P. Haskova, E. Attard, M. Germain, M.G. Martinoli, Cucurbitacin e has neuroprotective properties and autophagic modulating activities on dopaminergic neurons, Oxid. Med. Cell. Longev. 2014 (2014) 425496.
[23] C. Jacquot, B. Rousseau, D. Carbonnelle, I. Chinou, M. Malleter, C. Tomasoni, C. Roussakis, Cucurbitacin-D-induced CDK1 mRNA up-regulation causes proliferation arrest of a non-small cell lung carcinoma cell line (NSCLC-N6), Anticancer Res. 34 (2014) 4797–4806.
[24] Y. Kong, J. Chen, Z. Zhou, H. Xia, M.H. Qiu, C. Chen, Cucurbitacin E induces cell cycle G2/M phase arrest and apoptosis in triple negative breast cancer, PLoS One 9 (2014) e103760.
[25] M. Promkan, S. Dakeng, S. Chakrabarty, O. Bögler, P. Patmasiriwat, The effectiveness of cucurbitacin B in BRCA1 defective breast cancer cells,, PLoS One 8 (2013) e55732.
[26] M. Zhang, H. Zhang, C. Sun, X. Shan, X. Yang, J. Li-Ling, Y. Deng, Targeted constitutive activation of signal transducer and activator of transcription 3 in human hepatocellular carcinoma cells by cucurbitacin B, Cancer Chemother. Pharmacol. 63 (2009) 635–642.
[27] M.C. Recio, M. Prieto, M. Bonucelli, C. Orsi, S. Máñez, R.M. Giner, M. Cerdá-Nicolás, J.L. Ríos, Anti-inflammatory activity of two cucurbitacins isolated from Cayaponia tayuya roots, Planta Med. 70 (2004) 414–420.
[28] S. Yubero, L. Ramudo, M.A. Manso, I. De Dios, Mechanisms of dexamethasone-mediated chemokine down-regulation in mild and severe acute pancreatitis, Biochim. Biophys. Acta 1792 (2009) 1205–1211.