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A 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitor, 11b-0048, effectively suppresses the expression of 11β-HSD1 activated in cultured keratinocytes and in diabetic murine skin

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Abstract

Elevated level of active glucocorticoid (GC) deteriorates skin barrier function. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an NADPH-dependent enzyme converting inactive GC to active GC. Elevated active GC due to increased 11β-HSD1 expression might contribute to barrier impairment in aged skin and diabetic skin. We believe that the increase of 11β-HSD1 expression is a main cause of barrier abnormalities in diabetic skin and perform this study to elucidate the effect of a new 11β-HSD1 inhibitor. We compared it with a proven inhibitor in the cultured keratinocytes inducing typically 11β-HSD1 activation with dexamethasone treatment, UVB irradiation, and high glucose treatment, and the *db/db* mice as a type 2 diabetes murine model. In the cultured medium, cortisol, 11β-HSD1, and cytokines were measured. Also, in the *db/db* mice with a two-week application of 11β-HSD1 inhibitors, skin barrier function, HbA1c, corticosterone, 11β-HSD1, and cytokines were measured. In cultured keratinocytes, all concentrations and mRNA levels of cortisol, 11β-HSD1, and cytokines were decreased by both 11β-HSD1 inhibitors. In the *db/db* mice, both inhibitors improved skin barrier function and reduced serum level of HbA1c and skin expression of corticosterone, 11β-HSD1, and cytokines. A new 11β-HSD1 inhibitor, “11b-0048”, showed a significant inhibitory effect on the expression of 11β-HSD1 in keratinocytes activated by various conditions and diabetic skin.

INTRODUCTION

Glucocorticoid (GC) has been used for autoimmune diseases such as alopecia areata and chronic inflammatory dermatitis such as atopic dermatitis and psoriasis due to their potent immunosuppression and anti-inflammatory properties[1–5]. Synthesis of GCs starts from cholesterol and goes through several steps to become cortisol. Feedback from the hypothalamic-pituitary-adrenal (HPA) axis regulates GC level and acts on the skin, immune system, and adipose tissue[6].

The HPA axis indicates that physical or biological stress stimulates the hypothalamus to induce corticotropin-releasing hormone (CRH) by releasing stress signals from the brain, skin, and immune cells, and CRH stimulates the release of adrenocorticotropic hormone (ACTH) and the expression and processing of pro-opiomelanocortin (POMC) in the anterior pituitary gland. ACTH binds to the melanocortin 2 (MC-2) receptor in the zona fasciculata of the adrenal cortex and stimulates the transport of cholesterol into the mitochondria and consequently the production of cortisol. Cortisol is mainly produced in the adrenal gland, but it can be produced in various organs such as the immune system, skin, brain, and intestine[6,7].

The HPA axis plays an important role in psychological or physical stress and inflammatory conditions. GC produced through the central HPA axis is a key hormone that regulates the inflammatory response caused by internal or external stress[6–10]. Apart from the central HPA axis, the peripheral HPA axis system maintains homeostasis in response to continuous exposure to stress in the skin. Also, the active form of GC is produced in the skin through the peripheral 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1)

enzyme system[7,8]. 11β-HSD1 is expressed in epidermal keratinocytes, dermal fibroblasts, and hair follicles in human skin and converts cortisone, inactive GC, into cortisol, active GC[11–15].

GC bioavailability in the peripheral organs including the skin is dependent on two enzymes, 11β-HSD1 and 11β-HSD2[7,16]. 11β-HSD1 acts as a GC activator by reducing cortisone, an inactive GC, to cortisol, an active form, depending on nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide phosphate (NADP) levels. On the contrary, 11β-HSD1 oxidizes cortisol to cortisone to act as both an activator and inactivator, but it mainly acts as an active form of cortisol. On the other hand, 11β-HSD2 acts only as an oxidase to convert the hydroxy group at C11 of cortisol to ketone, thereby converting cortisol to cortisone[6].

Aging skin and the skin of patients with diabetes mellitus (DM) show similarities in barrier functions. In diabetic skin similar to aging skin, stratum corneum (SC) lipids such as ceramides, fatty acids, and cholesterol and lipid synthesis rate are decreased and the ability to restore skin barrier is also reduced compared to young healthy skin[17,18]. In the previous studies, we presented that elevated active GC due to increased 11β-HSD1 expression caused barrier impairment in aging skin, diabetic skin, and atopic dermatitis[19–21].

We believe that the increase of 11β-HSD1 expression is an important cause of barrier abnormalities in diabetic skin or aged skin (Supplementary Figure S1). Therefore, treatment of 11β-HSD1 inhibitors will be necessary to restore the skin barrier abnormalities in aged or diabetic skin. We performed this study to elucidate the effect of a new 11β-HSD1 inhibitor, 11b-0048, on the cultured keratinocytes and the type 2 diabetic murine skin.

RESULTS

Both 11β-HSD1 inhibitors decrease cortisol and 11β-HSD1 in normal HEKs. First, the expression of 11β-HSD1 was examined in HEKs that were treated with 1 μM of dexamethasone, 15 mJ UVB irradiation, and 26 mM/L of D-glucose. Thereafter, cortisol and 11β-HSD1 levels in the cultured medium were measured at the 24-hours interval. After twenty-four hours after treatment, 11β-HSD1 protein and mRNA were significantly increased along with cortisol level in the cultured medium, and a time-dependent increase was observed (Fig. 1A-C). The levels of cortisol and 11β-HSD1 in the cultured medium were higher when 15mJ UVB irradiation was performed than 1μM of dexamethasone or 26mM/L of D-glucose.

After additional treatment with both 11β-HSD1 inhibitors, the level of cortisol in the culture medium decreased over time, and the new 11β-HSD1 inhibitor showed a further decrease (Fig. 1A). Both the protein and mRNA of 11β-HSD1 decreased when treated with 11β-HSD1 inhibitor and showed a further decrease when treated with the new inhibitor, “11b-0048” (Fig. 1B, 1C). Detailed values are provided in Supplementary Table S1.

Both 11β-HSD1 inhibitors decrease IL-1α, IL-1β, and IL-6 in HEKs. Inflammatory cytokines (IL-1α, IL-1β, and IL-6) were measured in NHKs that were treated with 1μM of dexamethasone, 15mJ UVB irradiation, and 26 mM/L of D-glucose. Thereafter, cytokine levels in the cultured medium were measured at the 24-hours interval. After twenty-four hours after treatment, cytokine levels were significantly increased in the cultured medium, and a time-dependent increase was observed. The levels of cytokines in the cultured medium were higher when 15mJ UVB irradiation was performed than 1μM of dexamethasone or 26mM/L of D-glucose.

After additional treatment with both 11β-HSD1 inhibitors, the secretion of cytokines of HEKs decreased over time, and the new 11β-HSD1 inhibitor showed a further decrease (Fig. 2A-C). Detailed values are provided in Supplementary Table S2.

Both 11β-HSD1 inhibitors decrease HbA1c and SC corticosterone in db/db mice. Following *in vitro* study, we examined whether these results were the same in mice. We applied vehicle, a proven inhibitor, and a new inhibitor twice a day to *db/db* mice and wild-type mice for 14 days. On the 14th day after application, blood samples from the mice orbit were collected and HbA1c level was measured. HbA1c was higher in *db/db* mice than in the wild type. After both inhibitors were applied to the skin, HbA1c was statistically significantly decreased (Fig. 3A).

SC corticosterone was higher in *db/db* mice than in the wild type, and the protein and mRNA levels of 11β-HSD1 in the epidermis and dermis of skin samples obtained by skin biopsy were higher in *db/db* mice. Both inhibitors had significantly lower SC corticosterone than vehicle. However, SC corticosterone level did not differ between both inhibitors in *db/db* mice (14.01 ± 2.00 vs 13.32 ± 3.54, $p = 0.368$) and wild type (6.30 ± 1.22 vs 4.65 ± 2.75, $p = 0.029$) (Fig. 3B). Both 11β-HSD1 inhibitors decreased 11β-HSD1 protein and mRNA expression in the epidermis and dermis in both *db/db* mice and wild type (Fig. 3C-F). Detailed values are provided in Supplementary Table S3.

Both 11β-HSD1 inhibitors decrease epidermal cytokines in db/db mice. From the results of *in vitro* study, we hypothesized that 11β-HSD1 inhibitor could reduce inflammation in the skin. By skin biopsy, *db/db* mice and wild-type mice epidermis were obtained and inflammatory cytokine levels (IL-1α, IL-1β, TNF-α) were measured. Cytokine levels were higher in *db/db* mice than in the wild type. When both inhibitors were applied, cytokines showed a decreasing trend, but there was no statistically significant difference (Fig. 4A-D). Detailed values are provided in Supplementary Table S4.

Both 11β-HSD1 inhibitors improved the skin barrier recovery in db/db mice. The skin barrier function was measured after 14 days of application. The *db/db* mice had higher skin surface pH and SC hydration compared to the wild type. In addition, the *db/db* mice had lower basal TEWL and barrier recovery rates after D-Squame® disc tape stripping compared to the wild type.

There was no significant difference in skin surface pH between both inhibitors in *db/db* mice. Compared with the vehicle (7.37 ± 0.03) in the wild type, the proven inhibitor decreased the pH (7.17 ± 0.06, $p = 0.016$), and the new inhibitor increased the pH (7.44 ± 0.01, $p = 0.023$) (Fig. 4E). SC hydration was

decreased in both *db/db* mice and wild type. SC hydration was decreased in the new inhibitor than in the proven inhibitor (Fig. 4F). Basal TEWL was increased in both *db/db* mice and wild type, but the new inhibitor increased more than the proven inhibitor (Fig. 4G). The barrier recovery rate was increased in both *db/db* mice and wild type. In *db/db* mice, there was a statistically significant difference in recovery rates from proven inhibitors (30.38 ± 19.57 vs 93.91 ± 12.00, $p = 0.024$). However, in the wild type, the recovery rate increased more with the new inhibitor than with the proven inhibitor, but it was not statistically significant (40.61 ± 9.56 vs 60.10 ± 10.59 vs 104.94 ± 35.32) (Fig. 4H). Detailed values are provided in Supplementary Table S4.

DISCUSSION

GC in the skin increases the barrier function by promoting the differentiation of epidermal keratinocytes in the short term. On the contrary, long-term GC exposure paradoxically decreases the SC integrity and disrupts the skin barrier[9]. According to a previous study, the 11β-HSD1 enzyme system is activated in atopic dermatitis as well as in internal stress such as psychological stress, and external stress such as UVB irradiation, which induces chronic GC exposure for a long time[19,24]. This resulted in an abnormality of the skin barrier. Similarly, 11β-HSD1 is chronically increased in aged skin, which increases active GC, resulting in the abnormality of the skin barrier function. The skin barrier function in diabetic patients is similar to aged skin[18]. Although the precise mechanism underlying the abnormality of skin barrier function in hyperglycemic conditions has not yet been clarified, in our study, activation of the 11β-HSD1 enzyme system induced by hyperglycemic conditions and an increase in active GC were observed. Therefore, we thought that an increase in active GC due to activation of the 11β-HSD1 enzyme system in diabetic skin, similar to the aged, would affect the abnormality of the skin barrier function.

11β-HSD1 inhibitor was first proposed in a diabetes model[25] and is currently being studied in several disease models caused by inflammation, such as metabolic diseases and dementia[26–28]. In addition to these systematic disorders, 11β-HSD1 inhibitors have been studied in the skin. Several murine and human studies have reported that 11β-HSD1 inhibitor promotes fibroblast[13], improves the dermal contents[12,14,15], and modulates internal stress such as intrinsic aging and external stress such as UVB irradiation[20,24].

Our results showed the inhibitory effect of a new inhibitor, “11b-0048” on the 11β-HSD1 enzyme system in the skin. In *in vitro* study with high glucose treatment, mRNA of 11β-HSD1 showed a time-dependent increase, and in the murine study, the levels of SC cortisol and skin 11β-HSD1 were higher in *db/db* mice than in wild-type. The levels of inflammatory cytokines increased in HEKs treated with high glucose levels and in *db/db* mice. Also, the *db/db* mouse showed a lower skin barrier function than the wild-type. Overall, a GC increase in the skin was observed due to hyperglycemic conditions, which showed similarity with UVB irradiation and high corticosteroid treatment. This result showed that hyperglycemia, similar to known external/internal stress such as UVB irradiation and high corticosteroid treatment, stressed the skin.

In our previous study, we measured cortisol levels in the absence of any stress and found concentrations to be in the range of 1–2 ng/mL[24]. When 11β-HSD1 inhibitors were treated *in vitro*, the levels of 11β-HSD1 and cytokine were suppressed compared to the control. Similarly, when 11β-HSD1 inhibitors were applied in murine back, the levels of epidermal/dermal 11β-HSD1 and cytokines were suppressed. The application of 11β-HSD1 inhibitors increased the recovery of the skin barrier and reduced the abnormality of the barrier function. This result showed that 11β-HSD1 inhibition in the skin through topical 11β-HSD1 inhibitors modulated inflammatory cytokines and reduced abnormalities of SC integrity and skin barrier function even when continuously exposed to excessive systemic GC due to internal/external stress.

Although lower basal TEWL has been generally believed as a representative indicator of healthy barrier function, previous studies with aged and diabetic skin showed lower basal TEWL compared to healthy skin[18]. With the same context, this study showed higher basal TEWL after topical 11β-HSD1 inhibitors treatment than the vehicle. In *db/db* mice, the peripheral vascular system was impaired due to long-term high glucose[29,30]. Topical 11β-HSD1 inhibitor recovered peripheral vessels, which resulted in the increase of basal TEWL and the barrier recovery rate. Comprehensively, the abnormalities of skin barrier function were recovered by topical 11β-HSD1 inhibitors.

In this study, applying both 11β-HSD1 inhibitors reduced the HbA1c level. Long-term elevated GC causes metabolic syndrome, including insulin resistance[26,31]. Patients with metabolic syndrome are known to have a high activity of the 11β-HSD1 enzyme[32,33]. Therefore, the local GC concentration regulated by the peripheral 11β-HSD1 enzyme system is as emphasized as the GC regulated by the central HPA axis. For this reason, the 11β-HSD1 inhibitor is being studied as a promising treatment for metabolic diseases[28,34]. From this result, it could be seen once again that the regulation of the peripheral 11β-HSD1 enzyme system of the skin by the application of the topical 11β-HSD1 inhibitor can the affect whole body. Unlike *db/db* mice, there was no significant difference in skin barrier function of wild type between vehicle and 11β-HSD1 inhibitors. We consider that because the peripheral 11β-HSD1 enzyme system was not activated in the wild type, 11β-HSD1 inhibitors did not have a significant effect.

Our study had several limitations, First, our *in vitro* study used foreskin HEKs. Foreskin HEKs may not be representative of normal keratinocytes, as it is an immune-privileged organ. However, undifferentiated keratinocyte monolayers are not considered clinically relevant, hence the development of human epidermal equivalents[35]. Second, we were unable to treat cells with cortisone, measure the resulting cortisol levels +/- treatments and inhibitors, and normalize the results to viable cells. Third, for db/db mice, 9 mice were used for each group and 7 mice were used for wild type. Due to the small number of mice, the error was large and should be interpreted with caution. A larger number of animals is needed to confirm the trend of the experiment.

Despite these limitations, our study used a new 11β-HSD1 inhibitor, "11b-0048", along with "385581", as a proven 11β-HSD1 inhibitor. A new 11β-HSD1 inhibitor, “11b-0048” showed an equivalent or better 11β-HSD1 inhibitory effect than a proven 11β-HSD1 inhibitor.

11β-HSD1 inhibitors are undergoing multiple clinical studies for the treatment of type 2 diabetes mellitus, and several novel inhibitors are currently in development. 'MK-0916' did not show a significant improvement in fasting plasma glucose when compared to the placebo, but it did lead to modest improvements in HbA1C, weight, and blood pressure[36]. In a phase 2 randomized controlled trial of 103 patients with type 2 diabetes, treatment with 'BI 187004' did not produce significant results in parameters such as body weight or meal tolerance, glucose and lipid metabolism. A significant increase in weighted mean plasma glucose was observed at the 80 mg and 240 mg doses of BI 187004, but no significant change in fasting plasma glucose. Based on these results, further clinical development of BI 187004 was discontinued[34]. Furthermore, clinical development of BI 135585 and BI 163538 ceased after Phase I and II trials due to insufficient inhibition of the 11β-HSD1 target enzyme[37]. 'AZD4017' was evaluated in a randomized, double-blind, placebo-controlled Phase II study involving 93 patients diagnosed with non-alcoholic fatty liver disease/hepatitis. In patients with NASH and type 2 DM, the AZD4017 group showed improvement in liver steatosis compared to the placebo group[38]. 'TT-654' demonstrated an improvement in insulin resistance and obesity in Goto-Kakizaki rats, a DM model, by inhibiting adipose tissue and liver 11β-HSD1[39]. Consequently, 11β-HSD1 inhibitors are being investigated for a range of metabolic disorders, including type 2 DM, though clinical studies on their effects in the skin are limited. In an in vivo study, topical carbenoxolone applied to SKH1 mice fed water with corticosterone (100 μg/mL) increased barrier repair and wound healing[15]. In a Phase II clinical trial utilizing an oral 11β-HSD1 inhibitor to target diabetic skin, wound healing was promoted[40]. This study showed results similar to our study. They compared an oral 11b-HSD1 inhibitor, AZD4017, at a dose of 400 mg orally with placebo in patients with T2DM. The AZD4017 group showed more effective wound healing than the placebo group. Additionally, TEWL was 33% higher on average with AZD4017 on day 35. In addition, their study observed improvements in epidermal integrity and barrier recovery. Rather than using an oral inhibitor, we used a topical inhibitor, and the improvement in skin barrier function seen with the topical agent suggests that it could be a viable treatment alternative for T2DM patients who are likely to use multiple medications. As a result, it could be further developed in a larger clinical study.

In contrast to previous studies, our research employed a topical agent and demonstrated positive in vitro results for both UVB, high glucose, and dexamethasone treatment, as well as in *db/db* mice, showing that a novel 11β-HSD1 inhibitor is effective in treating diabetic skin. Therefore, "11b-0048" is a promising new 11β-HSD1 inhibitor that requires further evaluation and clinical trials.

In summary, active GC and 11β-HSD1 which play an important role in converting inactive GC into active GC increased in the skin with hyperglycemic conditions. 11β-HSD1 inhibitors improved skin barrier recovery and reduced serum levels of HbA1c and skin expression of corticosterone, 11β-HSD1, and cytokines. "11b-0048", a novel 11β-HSD1 inhibitor, showed a significant inhibitory effect on the expression of activated 11β-HSD1 in various conditions and diabetic skin, preventing and delaying skin damage for aged or patients with DM.

MATERIALS AND METHODS

11β-HSD1 inhibitors. We used "11b-0048", 1-[1-(1-adamantylacetyl)pyrrolidin-3-yl]-3,5-dimethyl-1H-pyrazole (ChemBridge Corp. San Diego, CA, USA), a new material designed and screened by COSMAX© (Sungnam, Korea) entrusted to the Bioinformatics and Molecular Design Research Center (BMDRC)©. A proven 11β-HSD1 inhibitor, "385581" (Merck, Readington Township, NJ, USA) was used as a positive control. We compared the effects of both 11β-HSD1 inhibitors on human keratinocytes and the type-2 diabetic murine model.

Cell culture. Primary human epidermal keratinocytes (HEKs) were obtained from healthy human neonatal foreskin (American Type Culture Collection, ATCC, Manassas, VA, USA). HEKs (50,000 cells/mL) were cultured in EpiLife™ medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with antibiotic-antimycotic and human keratinocyte growth supplement (Thermo Fisher Scientific) at 37°C under a 5% CO2. The cells were seeded on type-1 collagen-coated plates, then cultured for 3 days before use in experiments.

Toxicity study. To evaluate the cellular toxicity of 11b-0048, we dissolved it in DMSO and conducted a viability assessment using the MTT assay. 11b-0048 was dissolved in DMSO at concentrations of 0.25 μM, 0.5 μM, 1 μM, 5 μM, and 10 μM, respectively. After treating HEKs, we compared their viability 72 hours later. The viability results were as follows; 98.2% for DMSO alone, 97.9% for 0.25 μM, 96.3% for 0.5 μM, 90.6% for 1 μM, 61.1% for 5 μM, and 26.7% for 10 μM (Supplementary Figure S2). Based on these findings, we chose to use 1 μM of 11b-0048 for our experiments.

Activation of 11β-HSD1. After 3 days of cell seeding, we compared both 11β-HSD1 inhibitors in the HEKs inducing the activation of 11β-HSD1 with dexamethasone treatment, UVB irradiation, and high glucose treatment. The selective 11β-HSD1 inhibitors, "385581" and "11b-0048", were dissolved in dimethyl sulfoxide (DMSO) for topical application at a concentration of 1μM;

1) HEKs were treated with dexamethasone 1μM, and both inhibitors were treated.

2) HEKs were irradiated with UVB of 15mJ, and both inhibitors were treated.

3) The hyperglycemic condition was set for D-glucose at a concentration of 26 mmol/L, and both inhibitors were treated.

We measured supernatant for mRNAs and proteins of cortisol, 11β-HSD1, IL-1α, IL-1β, and IL-6 every 24 hours.

Preparation of animals. The Institutional Animal Care and Use Committee at Yonsei University Wonju College of Medicine approved all animal procedures (YWC-210409-1). And, this study was reported in accordance with ARRIVE guidelines. As a type 2 diabetic model, 4-week-old *db/db* mice (Daehan Biolink, Eumseong, Korea) were introduced, and a high-fat diet was performed until 14 weeks of age to form a hyperglycemic model. For *db/db* mice, 9 mice for each group and 7 mice for wild-type were used. The selective 11β-HSD1 inhibitors, "385581" and "11b-0048" were dissolved in DMSO for topical application at a concentration of 10μM. Each 100μl of both 11β-HSD1 inhibitors solutions was applied on the back of

14-week-old mice twice a day for 14 days. The vehicle solution was applied to groups 1 and 4, "385581", as a proven inhibitor, was applied to groups 2 and 5, and "11b-0048", as a new inhibitor, is applied to groups 3 and 6 (Supplementary Figure S3).

Measurement of skin barrier function. Skin barrier function was assessed as the basal trans-epidermal water loss (TEWL), SC hydration, and SC integrity [defined as changes in TEWL after four times of D-Squame® disc tape stripping] of the dorsal skin of mice[20,22,23]. The TEWL was quantified with a Tewameter TM 210 (Courage and Khazaka Electronic GmbH, Cologne, Germany). The calculation of recovery rate was as follows; [(TEWL at 0 h after barrier disruption - TEWL at 4 h after barrier disruption)/(TEWL at 0 h after barrier disruption - TEWL at baseline)] x 100. SC hydration was measured using a Corneometer CM 850 (Courage and Khazaka). Skin surface pH was measured with a pH meter (WTW, Weilheim, Germany).

Enzyme-linked immunosorbent assay (ELISA). The culture supernatant was centrifuged for 15 min at 1000 × g, 2–8 °C. The amount of cortisol in the samples was measured using a Human Cortisol ELISA Kit (CSB-E05111h, Cusabio Technology LLC, Houston, USA). According to the manufacturer's protocol, the expression of 11β-HSD1 was measured using the Human HSD11B1/HSD1B ELISA Kit (LS-F11298, LSBio, Seattle, USA). The amounts of cytokines were measured using a multiplex kit (LXSAHM, LABISKOMA, Seoul, Korea)[19].

In vitro, all experimental procedures were performed after 5% isoflurane inhalation anesthesia. HbA1c level was measured by ELISA (MBS2024955, MyBioSource, Inc., San Diego, USA). Stratum corneum was obtained by D-squame® tape stripping and the dorsal skin of mice was obtained by biopsy. D-squame disc tapes (CuDerm, Dallas, TX, USA) were attached to mice back skin to collect stratum corneum. Four strips were used to extract proteins. Strips were placed in 500 μL of lysis buffer, followed by vortexing and overnight incubation at 4°C. The strip was used to measure the cortisol of whole SC. The amount of SC corticosterone was measured using a Cortisol ELISA Kit (CSB-E05113m, Cusabio Technology LLC). The expression of 11β-HSD1 in the epidermis and dermis was measured using the HSD11B1/HSD1B ELISA Kit (AE39390MO, Abebio, Wuhan, China), according to the manufacturer's protocol. The amounts of cytokines were measured using a multiplex kit (LXSARM, LABISKOMA)[24].

Quantitative real-time polymerase chain reaction (RT-PCR). Total RNA was isolated from HEKs using QuantiTect Reverse Transcription Kits (Qiagen, Hilden, Germany). The product was reverse-transcribed into first-strand complementary DNA (cDNA), then 11β-HSD1 expression was measured using QuantiFast SYBR Green PCR Master Mix (Qiagen) as described by the manufacturer. Messenger RNA was quantified by RT-PCR using a 7900HT FAST Real-Time PCR System (Applied Biosystems, Franklin Lakes, NJ, USA). Primer concentrations were first optimized to avoid non-specific binding of primers. After the PCR, dissociation curves were analyzed to verify the specificity of the amplification products. The mRNA was normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the following sequence-specific primers were designed: GAPDH sense, 5 -TTGATTTTGGAGGGATCTCG-3 and antisense, 5 -GAGTCAACGGATTTGGTCGT-3 ; 11β-HSD1, sense: 5 -TCTCCTCTCTGGCTGGGAAAG-3 , antisense: 5 -

GAACCCATCAAAGCAAACTTG-3 . The conditions for thermal cycling were as follows: denaturation for five min, followed by 45 cycles of amplification at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 76°C for 30 s[19,20,24].

Statistical analysis. The data are expressed as mean ± standard error (SE). The unpaired Student's t-tests and repeated-measures analysis of variance were performed to determine the level of significance of differences between the sample mean. Statistical analyses were performed using GraphPad Prism software (San Diego, CA, USA). Values with $p < 0.05$ were considered statistically significant.

Declarations

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AUTHOR CONTRIBUTIONS

Lee Ju Yeong: Conceptualization, formal analysis, investigation, methodology, resources, validation, visualization, writing (original draft preparation, review, and editing)

Heo YW: Conceptualization, formal analysis

Hwang HJ.: Investigation, formal analysis

Kim E.: Investigation

Lee Jee-Young: Project administration, resources, validation

Kang S.: Project administration, resources, validation

Choi EH: Conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing (review and editing)

DATA AVAILABILITY

Data generated or analysed during this study are included in this published article and its supplementary information files.

IRB APPROVAL STATUS

This study was approved by the Institutional Animal Care and Use Committee at Yonsei University Wonju College of Medicine approved all animal procedures (YWC-210409-1) and a waiver of informed consent was granted owing to the deidentified data used.

CONFLICT OF INTERESTS

The patent for the new 11β-HSD1 inhibitor, 11b-0048, is in COSMAX©.

Seunghyun Kang is an employee of Cosmax©.

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Figures

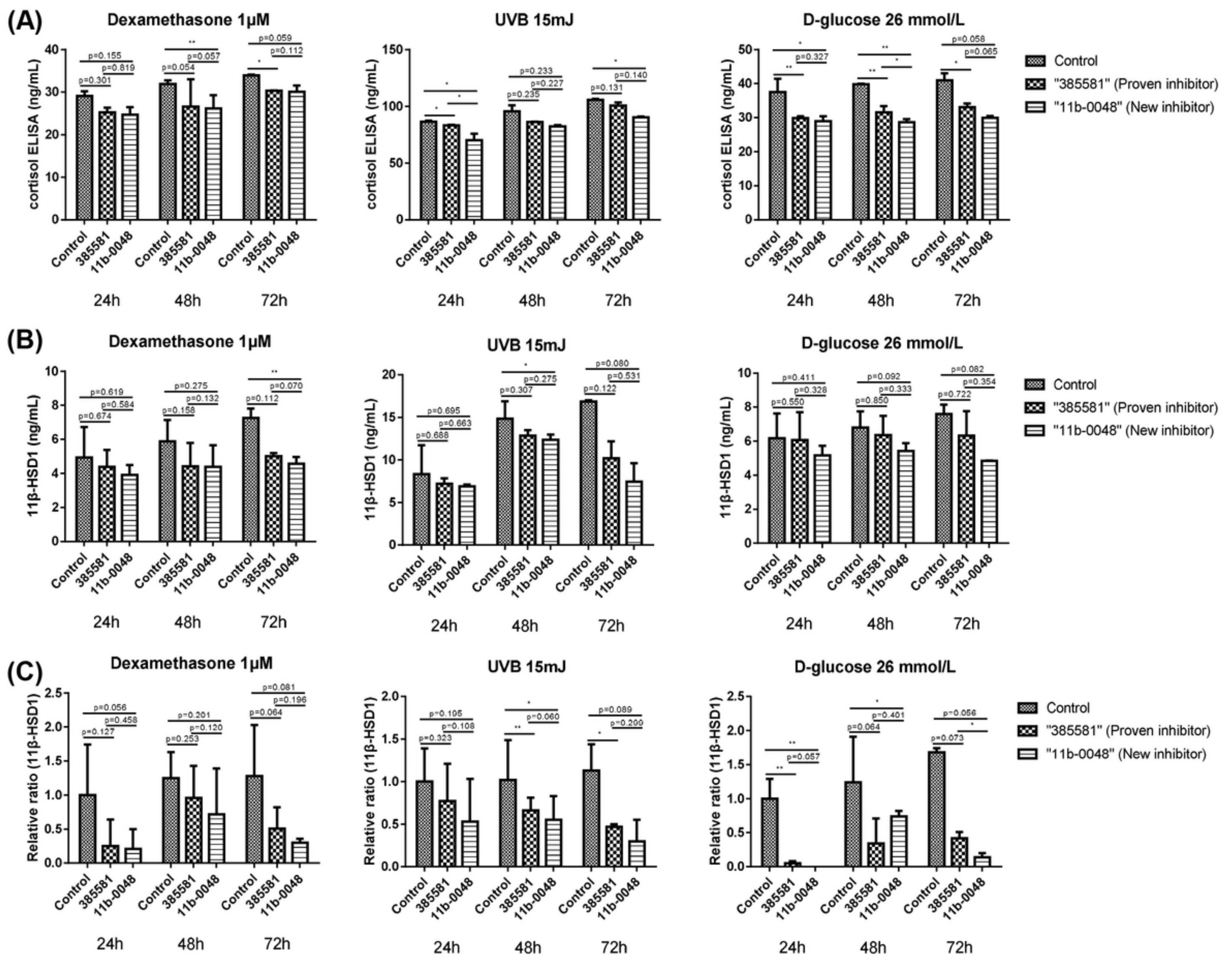

Figure 1

Levels of cortisol and 11β-HSD1 in the cultured keratinocyte after the treatment with both 11β-HSD1 inhibitors.

In experiments measured at 24-hour intervals, time-dependent increases in the level of cortisol and 11β-HSD1 were observed in dexamethasone, UV radiation, and high glucose-treated conditions. (A) After additional treatment with a new 11β-HSD1 inhibitor and a proven inhibitor, cortisol levels in the culture medium decreased over time, and the new inhibitor showed a further decrease. (B), (C) 11β-HSD1 protein level and mRNA in the culture medium decreased over time in both 11β-HSD1 inhibitor treatments but showed a further decrease in the new inhibitor. *, p-value < 0.05, **, p-value < 0.01. 11β-HSD1, 11β-Hydroxysteroid dehydrogenase type 1; UV, ultra-violet.

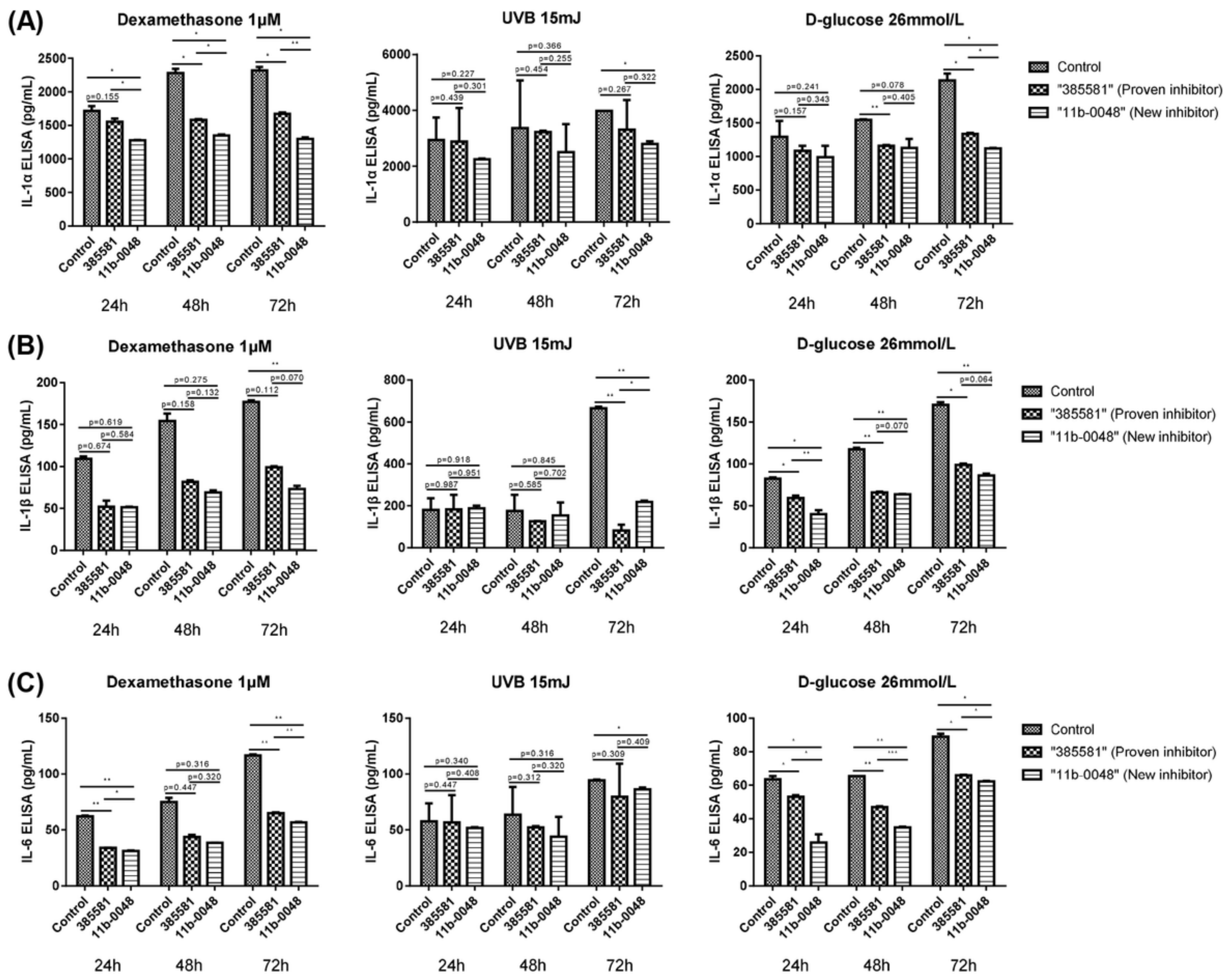

Figure 2

Cytokine levels in the cultured keratinocyte after the treatment with both 11β-HSD1 inhibitors. In experiments measured at 24-hour intervals, a time-dependent increases in the levels of cytokines were observed in dexamethasone, UV radiation, and high glucose-treated conditions. The secretion of cytokines IL-1α, IL-1β, and IL-6 in the culture medium was also decreased in both 11β-HSD1 inhibitor treatments but showed a further decrease with the new inhibitor. *, p-value < 0.05, **, p-value < 0.01, ***, p-value <0.001. 11β-HSD1, 11β-Hydroxysteroid dehydrogenase type 1.

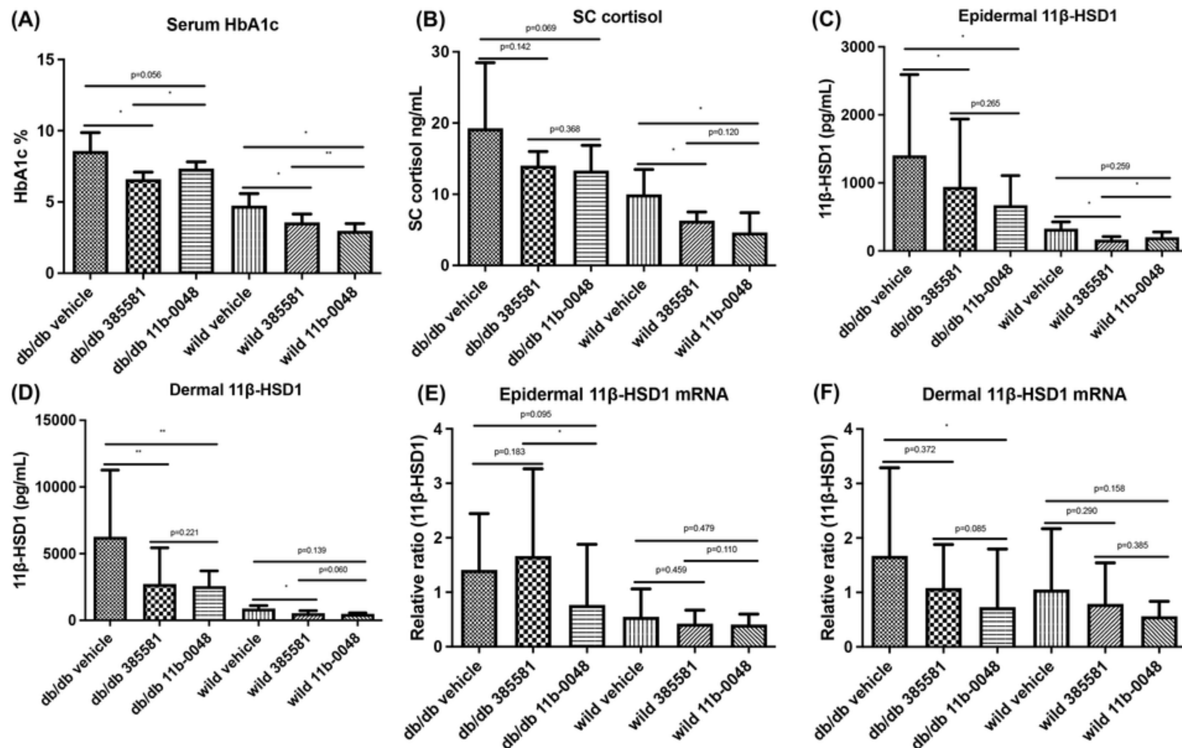

Figure 3

Levels of serum HbA1c, SC corticosterone and skin 11β-HSD1 in the diabetic murine model after the treatment with both 11β-HSD1 inhibitors. When comparing the *db/db* mice and the wild-type mice, the *db/db* mice had higher levels of serum HbA1c, SC corticosterone, and 11β-HSD1. Both 11β-HSD1 inhibitors reduced not only HbA1c level but also SC corticosterone and 11β-HSD1 levels in the epidermis and dermis in diabetic mice. *, p-value < 0.05, **, p-value < 0.01. 11β-HSD1, 11β-Hydroxysteroid dehydrogenase type 1; SC, stratum corneum.

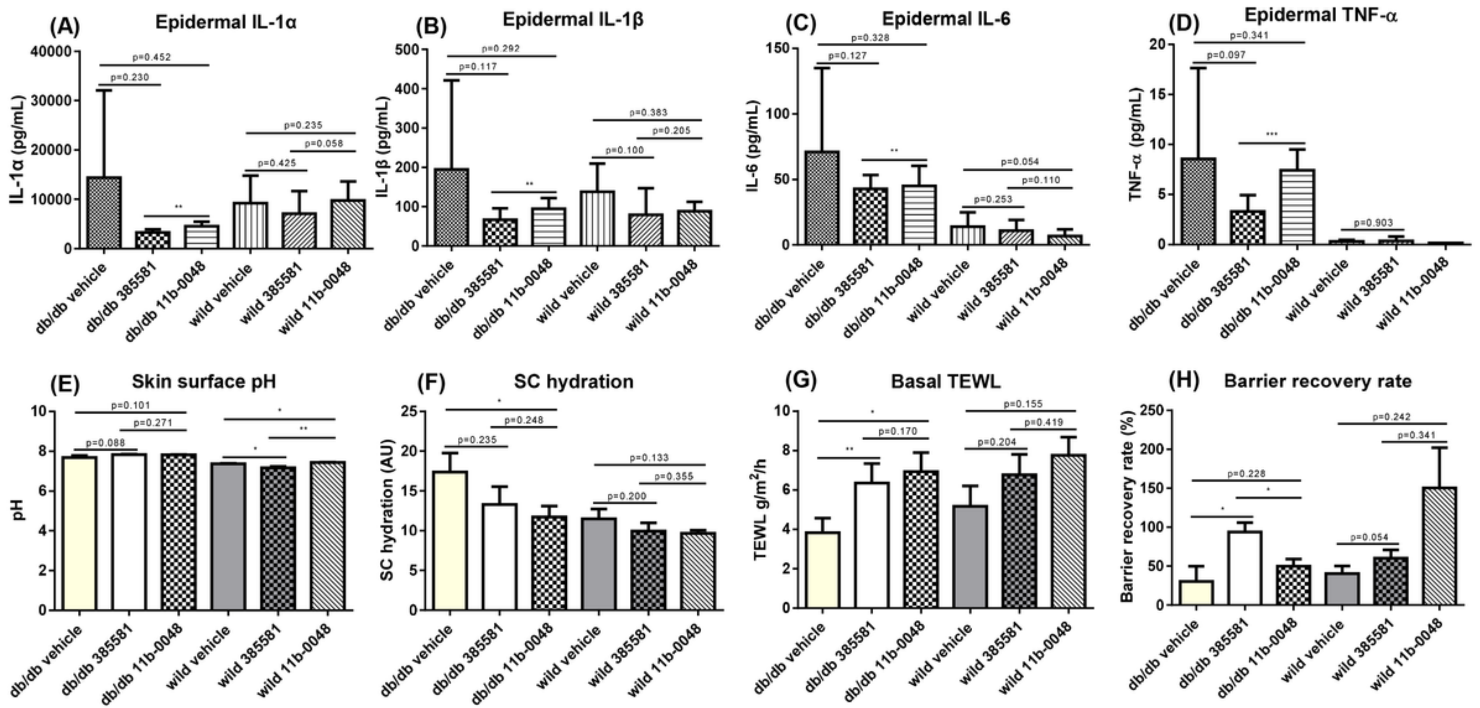

Figure 4

Epidermal cytokine levels and parameters of skin barrier function in the diabetic murine epidermis after the treatment with both 11β-HSD1 inhibitors. (A-D) When comparing the *db/db* mice and the wild-type mouse, *db/db* mouse had higher levels of the cytokine. In diabetic mice, the secretion of cytokines such as IL-1α, IL-1β, IL-6, and TNF-α was also decreased in both 11β-HSD1 inhibitor treatments. (E-H) When comparing the *db/db* mice and the wild-type mice, skin barrier functions were poor in the *db/db*mice. When both 11β-HSD1 inhibitors were applied to the skin in diabetic mice, there was no difference in skin surface pH. SC hydration was decreased, basal TEWL was increased, and the barrier recovery rate accelerated compared to the vehicle. *, p-value < 0.05, **, p-value < 0.01, ***, p-value <0.001. 11β-HSD1, 11β-Hydroxysteroid dehydrogenase type 1; SC, stratum corneum; TEWL, trans-epidermal water loss.

Supplementary Files

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