Hyperglycemia-activated 11β-hydroxysteroid dehydrogenase type 1 increases endoplasmic reticulum stress and skin barrier dysfunction

Young Bin Lee

The Graduate School
Yonsei University
Department of Medicine

Hyperglycemia-activated 11β-hydroxysteroid dehydrogenase type 1 increases endoplasmic reticulum stress and skin barrier dysfunction

Directed by Professor Eung Ho Choi

The Doctoral Dissertation
Submitted to the Department of Medicine,
the Graduate School of Yonsei University
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

Young Bin Lee

December 2024

This certifies that the Doctoral Dissertation of Young Bin Lee is approved.

_____

Thesis Supervisor: Eung Ho Choi

_____

Thesis Committee Member #1: Choon Hee Chung

_____

Thesis Committee Member #2: Seung Kuy Cha

_____

Thesis Committee Member #3: Seung Phil Hong

_____

Thesis Committee Member #4: Solam Lee

The Graduate School

Yonsei University

December 2024

감사의 말씀

무엇보다 저에게 의사 그리고 연구자로서의 삶과 덕목을 알려주신 최응호 교수님께 감사의 말씀을 드립니다. 이 연구는 최응호 교수님의 지도하 선행 연구를 맡아온 여러 선배님들의 지혜와 노고가 있었기에 가능하였습니다. 이 연구가 박화영 선생님과 김재홍 선생님의 '당뇨병에서의 피부 장벽 기능 연구'라는 주제와 이누리 선생님, 최승재 선생님, 김범준 선생님 그리고 이한일 선생님의 '피부의 당질코르티코이드 조절 연구'라는 주제의 맥락을 이어나가는 연구라는 점에서 개인적으로 뿌듯함과 감사함을 느낍니다.

바쁘신 와중에 제 학위의 심사를 맡아 매번 조언을 아끼지 않으신 정춘희 교수님, 차승규 교수님, 홍승필 교수님 그리고 이솔암 교수님께 감사드립니다. 본 연구를 물심양면 도와준 황현지 연구원 선생님, 김은정 연구원 선생님, 그리고 임성하 선생님께 다시한번 감사의 말씀을 드립니다. 뿐만 아니라 피부과학에 뜻을 둘 수 있도록 교실 안팎으로 가르침을 주신 이원수 교수님을 비롯한 은사님들과, 같이 배워가며 성장할 수 있도록 곁에서 도와준 김충혁 선생님을 비롯한 동기 및 후배 선생님들께도 진심으로 감사드립니다.

끝으로 학문에 뜻을 두고 걸어올 수 있도록 키워주시고 응원해주신 부모님(아버지 이인식과 어머니 박현미)께 한없이 그리고 원없이 감사드립니다. 그리고 이 길을 앞으로도 포기하지 않고 걸어갈 수 있음은 제 삶의 전부인 아내 김지희와 딸 이해이 덕분입니다. 지칠 때마다 항상 위로가 되어주는 동생 이유빈과 종우회 친구들에게 감사의 말씀을 전하며 마치고자 합니다.

2024 년 12 월

이영빈 올림

LIST OF CONTENTS

LIST OF FIGURES

ABSTRACT

Hyperglycemia-activated 11β-hydroxysteroid dehydrogenase type 1 increases endoplasmic reticulum stress and skin barrier dysfunction

Young Bin Lee

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Eung Ho Choi)

The diabetes mellitus (DM) skin shows skin barrier dysfunction and skin lipid abnormality, similar to conditions induced by systemic or local glucocorticoid excess and aged skin. Inactive glucocorticoid (GC) is converted into active glucocorticoid by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). Hyperglycemia in DM and excessive GC are known to increase endoplasmic reticulum (ER) stress. We hypothesized that hyperglycemia affects systemic GC homeostasis and that the action of skin 11β-HSD1 and GC contributes to increased ER stress and barrier defects in DM. We compared 11β-HSD1, active GC, and ER stress between hyperglycemic and normoglycemic conditions in normal human keratinocytes and db/db mice. 11β-HSD1 and cortisol

increased with time in keratinocyte culture under hyperglycemic conditions. 11β-HSD1 siRNA-transfected cells did not induce cortisol elevation in hyperglycemic condition. The production of 11β-HSD1 and cortisol was suppressed in cell culture treated with an ER stress-inhibitor. A reduction in ER stress was observed in cell cultures treated with the 11β-HSD1 inhibitor, suggesting a mutually regulatory relationship between ER stress and the 11β-HSD1/GC axis. The 14-week-old db/db mice showed higher stratum corneum (SC) corticosterone, and skin 11β-HSD1 levels than 8-week-old db/db mice. Topical 11β-HSD1 inhibitor application in db/db mice decreased SC corticosterone levels and improved skin barrier function. Hyperglycemia in DM may affect systemic GC homeostasis, activate skin 11β-HSD1, and induce local GC excess, which increases ER stress and adversely affects skin barrier function.

Keywords: Hyperglycemia; 11β-hydroxysteroid dehydrogenase type 1; Keratinocyte; Endoplasmic reticulum stress; Glucocorticoid; db/db mice

Introduction

The skin plays a vital role in maintaining homeostasis and protecting against stressful conditions through its complex interactions of mediators, thereby performing various neuroendocine functions[1-3]. In particular, the skin, which is responsible for extra-adrenal and extra-gonadal steroidogenesis, requires a precise balance of glucocorticosteroids (GCs) to maintain proper innate immunity and skin barrier function[4,5].

Local GC excess derived from dyshomeostasis of steroidogenesis in the skin leads to decreased skin thickness, and decreased collagen density in the dermis. The subsequent weakening of skin barrier function and delayed wound healing are consistent with the characteristics of cutaneous adverse effects caused by long-term topical GC use and the aging skin of the elderly[6-8]. Thus, the aging skin and prolonged use of topical GCs are considered local GC excess conditions.

The role of 11β-hydroxysteroid dehydrogenase (11β-HSD) in GC homeostasis has been highlighted. 11β-HSD type 1 (11β-HSD1) converts inactive GC to active GC, and 11β-HSD type 2 reverses this conversion[5]. The 11β-HSD is located at the membrane of endoplasmic reticulum (ER) and is strongly expressed in the suprabasal layer of epidermis[9]. The 11β-HSD expression in the skin (especially keratinocytes) is regulated by various stimuli, such as aging and UV exposure[9,10]. The 11β-HSD1 increase in the skin promotes the local steroidogenic pathway and inhibits the proliferation of keratinocytes[11,12].

Patients with diabetes mellitus (DM) have a higher prevalence of xerosis and delayed wound healing due to compromised skin barrier function compared to the general population[13,14].

In our previous studies, we described the skin of patients with DM as an acceleration of aging due to impaired skin barrier function in the patients with DM, similar to that of aging skin[13,15].

ER stress activates an unfolded protein response to exert protective effects on normal ER function triggered by various extrinsic and intrinsic factors, including UV irradiation and oxidative stress[16,17]. In particular, the relationship between ER stress and advanced glycation end-product (AGE) and hyperglycemia has been reported[18,19]. Glucose is essential for energy production through the electron transport chain, during which reactive oxygen species (ROS) are generated as byproducts. In a high-glucose environment, excessive production of ROS leads to overburdening of antioxidant processes, ultimately triggering oxidative stress[20]. The increase in ER stress under hyperglycemic conditions has a pervasive impact on insulin signaling in adipocytes, hepatocytes, and β-cells. Notably, it exacerbates insulin resistance via the IRE1-JNK-IRS-1 signaling pathway[21]. Severe or prolonged ER stress leads to apoptosis signaling beyond cellular dysfunction, and C/EBP is known to be one of the factors mediating this pathway[22,23].

However, the exact mechanism by which the increase in serum AGE induced by long-standing hyperglycemic conditions suppresses the proliferation of epidermal keratinocytes and eventually deteriorates skin barrier function remains unclear.

Therefore, as we hypothesized that the presence of DM would affect GC homeostasis, we investigated whether hyperglycemic conditions induce the dysregulation of hypothalamus-pituitary-adrenal (HPA) axis and further elevation of cortisol. In addition, considering the role of 11β-HSD1 on keratinocytes, we aimed to determine the impact of 11β-HSD1 in local GC excess under in vitro and in vivo hyperglycemic conditions.

Materials and Methods

1. In vitro study

1.1. Tissue preparation.

Normal human epidermal keratinocytes (NHK, 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 5% CO2. Third passage keratinocytes were utilized for the experiment. After 3 days of cell seeding, normoglycemic and hyperglycemic conditions were established by D-glucose treatment. As described in the literature, normoglycemic and hyperglycemic conditions were set for D-glucose at concentrations of 6 mmol/L and 26 mmol/L, respectively[48-50]. After 3 days of D-glucose treatment, the amounts of protein and mRNA were detected in the culture medium. The reagents used for intervention in cell culture were as follows; The selective 11β-HSD1 inhibitor, CAS 1009373-58-3 (Merck & Co., Kenilworth, NJ, USA) and 4-Phenyl butyric acid (4-PBA), P21005 (Merck & Co., Kenilworth, NJ, USA). All methods were carried out in accordance with relevant guidelines and regulations.

1.2. Transfection with siRNA

A day prior to transfection, the cells were seeded on type-1 collagen-coated plates. The cells were transfected with 50 nM 11β-HSD1 siRNA or control siRNA (Bioneer, Daejeon, Korea) using a mixture of Opti-MEM and Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA). The culture medium was replaced 6 h later. Cells were then used for the experiments 48 h after transfection.

1.3. Real-time reverse transcription PCR (RT-PCR) of mRNA

Total mRNA was isolated from NHKs using QuantiTect Reverse Transcription Kits (Qiagen, Hilden, Germany). The product was then reverse-transcribed into first-strand complementary DNA (cDNA). Approximately 60 ng of cDNA was used as the template for each reaction. The housekeeping gene GAPDH was used as an internal reference to normalize the data, accounting for any differences in sampling. All PCR reactions were performed in triplicate, and the results were expressed as the mean of values obtained from three separate experiments. Amplification of samples were carried out using the primers under the following conditions: initial denaturation at 95 °C for 15 min, followed by 45 cycles of 95 °C for 15 s and annealing/extension 60 °C for 1 min. The following primers were designed: C/EBPα, sense: 5′-TCAGCCGATATCAACACTTG-3′, antisense: 5′-AGTATCCGAGCAAAACCAAA-3′; C/EBPβ, sense: 5′-GACAAGCACAGCGACGAGTA-3′, antisense: 5′-AGCTGCTCCACCTTCTTCTG-3′; CHOP, sense: 5′-AAGGCACTGAGCGTATCATGT-3′, antisense: 5′-TGAAGATACACTTCCTTCTTG AACA-3′; Filaggrin, sense: 5′-TGAAGCCTATGACACCACTGA-3′, antisense: 5′-

TCCCCTACGCTTTCTTGTCCT-3′; Involucrin, sense: 5′-TCCTCCAGTCAATACCCATCAG-3′, antisense: 5′-CAGCAGTCATGTGCTTTTCCT-3′; Loricrin, sense: 5′-AGTGGACTGCGTGAAGAC-3′, antisense: 5′-GCCAGAACCGCTGCTACC-3′.

1.4. Enzyme-linked immunosorbent assay (ELISA)

The culture supernatant was centrifuged for 15 min at 1000 × g, 2–8 ℃. The amount of cortisol in the samples was measured using a Human Cortisol ELISA Kit (Cusabio, Houston, TX, USA) according to the manufacturer's protocol. The expression of 11β-HSD1 was measured using the Human HSD11B1/HSD1B ELISA Kit (LSBio, Seattle, WA, USA). In addition, the LS-F8872 CHOP ELISA Kit (LSBio, Seattle, WA, USA), ab190807 RAGE ELISA Kit (Abcam, Boston, MA, USA), LS-F32426 LSBio C/EBPα ELISA Kit (LSBio, Seattle, WA, USA), MBS2511196 C/EBPβ ELISA Kit (MyBioSource, San Diego, CA, USA) were used.

2. In vivo study

The research protocol for experiment using animal was approved by the Animal Ethical Committee (Institutional Animal Care and Use Committee, IACUC, YWC-200217-1) of the Yonsei University Wonju College of Medicine, Wonju, Korea. All methods are reported in accordance with ARRIVE guidelines.

2.1. Animal preparation.

Four-week-old db/db female mice (BKS.Cg-Dock7m +/+ Leprdb/db/J) (n = 15), which were used as an animal model of DM, and C57BL/6J female mice (n = 15), which served as a control strain, were supplied by The Jackson Laboratory (Bar Harbor, ME, USA). The 11β-HSD1 inhibitor was dissolved in DMSO for topical application at a concentration of 100 μM 36. DMSO was used as vehicle control. Vehicle and topical 11β-HSD1 inhibitors were applied twice a day to the dorsal surface of the mice for two weeks. All animals were housed in a standard environment with the temperature maintained at 22 ± 0.5 ℃, relative humidity at 60 ± 5%, and a 12-h/12-h light/dark cycle. After a one-week acclimatization period, the mice were fed a high-fat diet. In db/db mice, it is known that the degree of glucose intolerance develops with maturation; hyperglycemia usually occurs after 5 weeks, and the insulin concentration peaks at 3 months, with a maximum body weight of approximately 60 g. Accordingly, 8-week-old db/db mice were selected as the intermediate stage for the development of DM, and 14-week-old db/db mice were set as fully developed DM conditions.

Because we investigated the hormones involved in the HPA axis, we maintained a stress-minimized (handling-minimized) environment. Thus, one cage was used for each mouse. As db/db mice developed DM as they matured, the onset of symptoms due to polyuria and polyphagia became more frequent. Therefore, frequent changes in the cage and dietary supplementation were performed.

A constant day-night cycle and time of topical reagent application were maintained during the breeding period. Considering the circadian rhythm of mice, the experiment (collection of blood and specimen under anesthesia) was conducted between 1:00 PM–3:00 PM, when the variation of cortisol was relatively lower compared to the early phase of the light-cycle[38,56,57]

2.2. Real-time reverse transcription PCR (RT-PCR) of mRNA

Isolation of epidermis. Skin samples were placed on the epidermis-side downward on Petri dishes, and subcutaneous fat was removed with a scalpel. Skin samples were then placed on the epidermis side up onto 10 mM EDTA in phosphate buffered saline and incubated at 37 °C for 30 min to separate the epidermis from the dermis. Following incubation in EDTA, the epidermis was scraped off with a scalpel, and total RNA was extracted.

The following primers were designed: C/EBPα, sense: 5′-TGGACAAGAACAGCAACGAGTAC-3′, antisense: 5′-GCAGTTGCCCATGGCCTTGAC-3′; C/EBPβ, sense: 5′-TTCTACTACGAGCCCGACTGCC-3′, antisense: 5′-CAGCTTGTCCACCGTCTTCTTG-3′;

CHOP, sense: 5′- CCACCACACCTGAAAGCAGAA-3′, antisense: 5′-
AGGTGCCCCCAATTTCATCT -3′.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The serum level of AGE was determined using a mouse AGE ELISA kit (Uscn Life Science Inc., Wuhan, China), while the serum levels of ACTH and corticosterone were determined using a mouse ACTH ELISA kit (ALPCO Diagnostic, Salem, NH, USA) and a mouse corticosterone ELISA kit (Enzo Life Sci., Plymouth Meeting, PA, USA), respectively. The skin level of 11β-HSD1 was estimated using a mouse 11β-HSD1 kit (Wuhan Abebio Science Co., Wuhan, China). To detect serum levels of AGE, ACTH, and corticosterone, pre-dialysis blood samples were obtained after 12 h of overnight fasting.

2.4. Assessment of skin barrier function

Hair shaving was performed 3 days before the experiment under anesthesia (inhalant anesthesia with 2.5% sevoflurane) without interruption of the day-night cycle. TEWL was measured using a Tewameter (TM 300; Courage & Khazaka, Cologne, Germany), and SC hydration was measured using a Corneometer (CM 825; Courage & Khazaka). Basal TEWL was measured on the dorsal surface of the mice. Barrier recovery was determined by measuring TEWL immediately after and at 3 h after acute barrier disruption by tape stripping. The recovery rate was calculated as described

previously. SC integrity was determined by measuring TEWL after four sequential strippings with D-squame® discs (CuDerm Corporation, Dallas, TX, USA)[13,15].

2.5. Quantification of stratum corneum (SC) cortisol

SC samples of the dorsal skin were collected from all mice by stripping off D-Squame® discs from their skin. The samples were placed in 500 μL of lysis buffer, vortexed, and incubated overnight at 4 °C. Cortisol levels in the collected protein extracts were measured using the corresponding ELISA kits (Aviva System Biology Corp., San Diego, CA, USA)[41]

3. Statistical analysis

All data are expressed as mean ± standard error (SE). Statistical analyses were performed using unpaired Student's t-tests and one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparison.

Results

1. Hyperglycemic condition induced the elevation of levels of 11β-HSD1, cortisol, and mRNA of RAGE, and the ER stress

Hyperglycemia resulted in a gradual decrease in cell viability over time. The viability was <80% at 24 h in NHKs under hyperglycemic conditions (Fig. 1a).

Protein and mRNA levels were compared between normoglycemic and hyperglycemic conditions in cell culture. The 11β-HSD1 and cortisol levels increased with time in NHK under hyperglycemic conditions. In contrast, a quantitative increase in 11β-HSD1 and cortisol with time was not observed in NHK under normoglycemic conditions. The difference in the amount between the normoglycemic and hyperglycemic conditions at each time point was significant (Fig. 1b, 1c).

The mRNA levels of epidermal receptor of AGE (RAGE) and CHOP (terminal marker of ER stress) showed a steep increase in the hyperglycemic condition in the first 24 h compared to the normoglycemic condition, which then decreased after 48 h. Under hyperglycemic conditions, a slight rebound was observed at 72 h. Significant changes in the hyperglycemic condition were not observed under normoglycemic conditions (Fig. 1d, 1e). The mRNA levels of C/EBPα and C/EBPβ showed a similar curve in the hyperglycemic condition. However, under normoglycemic conditions, the change with time was relatively weak (Fig. 1f, 1g).

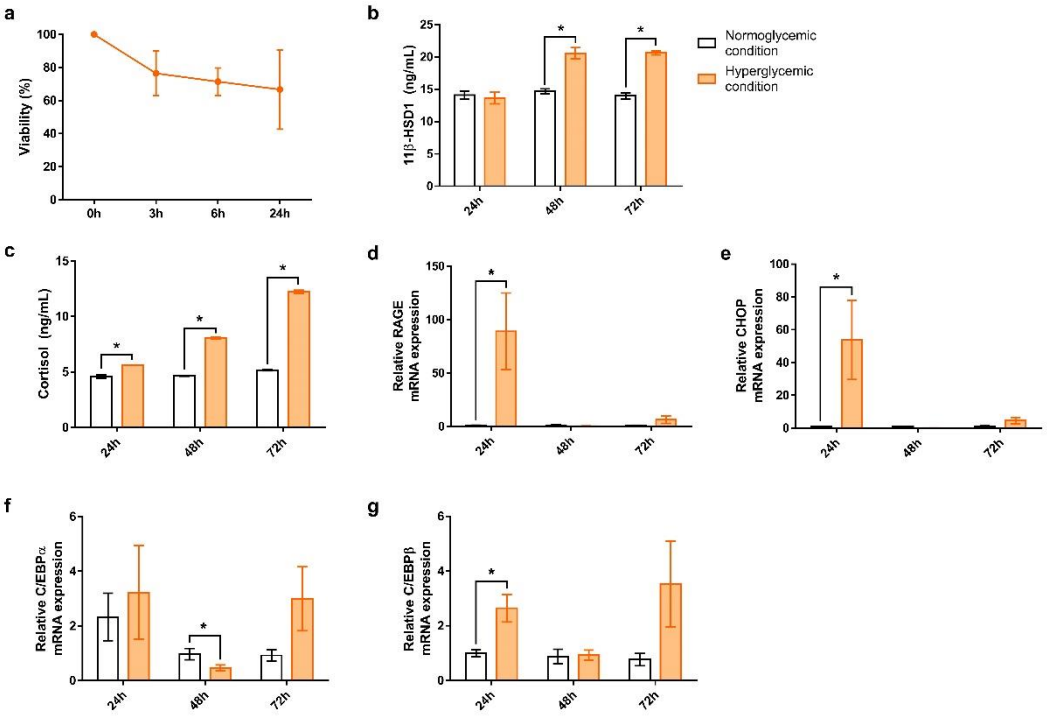

Figure 1. Hyperglycemic condition induced the elevation of the levels of 11β-HSD1, cortisol, and mRNA of RAGE, and the ER stress

Cell viability of normal human keratinocytes (NHKs) was evaluated up to 72 h at 24-h intervals after glucose treatment (a). 11β-HSD1 levels in NHKs under hyperglycemic conditions (treated with 26 mmol/L of D-glucose) were significantly higher than those in normoglycemic conditions (treated with 6 mmol/L of D-glucose) (b). A time-dependent increase in cortisol was observed in the hyperglycemic condition; no increase was observed in the normoglycemic condition (c). The RAGE and CHOP mRNA expression significantly increased at 24 h and decreased from 48 h in the hyperglycemic condition; the change under normoglycemic conditions was insignificant (d, e). The C/EBP mRNA expressions were high at 24 h, decreased at 48 h, and increased at 72 h in hyperglycemic conditions; these patterns were unapparent under normoglycemic conditions (f, g).

GAPDH was used as an internal control (d–g). Bars indicate the mean ± SE (N=3; *p<0.05, Student's t-test).

2. 11β-HSD1 knockdown suppressed the elevation of cortisol levels with a partial decrease of ER stress in the hyperglycemic condition

The mRNA and protein levels were compared between hyperglycemic and normoglycemic conditions in the 11β-HSD1 siRNA-transfected NHK. Following transfection, the amount of 11β-HSD1 decreased, and this decrease was more pronounced under hyperglycemic conditions than under normoglycemic conditions (Fig. 2a). In the siRNA-transfected cells, cortisol levels tended to decrease with time (Fig. 2b). Notably, this tendency was far different from the time-dependent increase of cortisol in non-transfected NHKs under hyperglycemic conditions (Fig. 1c). At all times, the amount of cortisol was significantly lower in the hyperglycemic condition than in the normoglycemic condition, indicating that the action of 11β-HSD1 is more pronounced in hyperglycemia than in normoglycemia.

In the siRNA-transfected cells, RAGE, CHOP, and C/EBP mRNA showed an increase at 24 h, the lowest at 48 h, and re-increase at 72 h, similar to the non-transfected condition (Fig. 2c–2h). Although the expression pattern in the transfected condition was similar to that shown in the non-transfected condition, the absolute value of RAGE and CHOP mRNA expression in the hyperglycemic condition was relatively lower in the 11β-HSD1 siRNA-transfected condition than in the non-transfected or scrambled siRNA control (Fig. 2c–2f). Therefore, the 11β-HSD1 knockdown in the hyperglycemic condition could suppress the elevation of cortisol levels with decrease in ER stress.

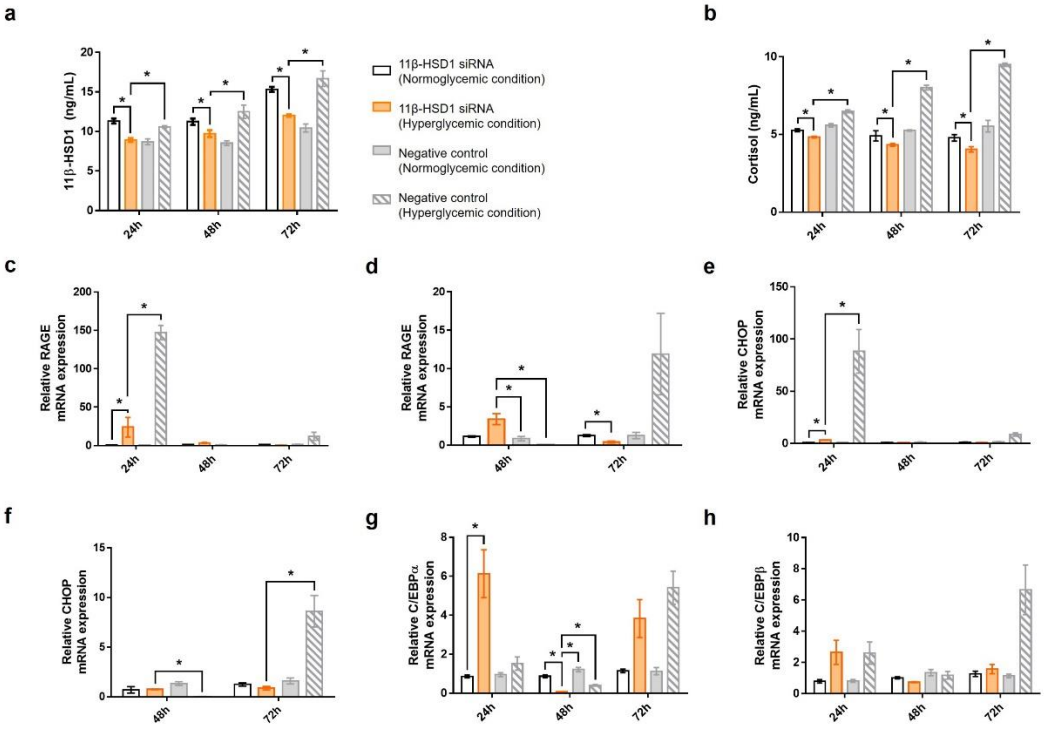

Figure 2. No increase in cortisol level and a partial decrease in ER stress were observed in normal human keratinocytes transfected with 11β-HSD1 siRNA

Normal human keratinocyte cultures were transfected with 11β-HSD1 siRNA, and 11β-HSD1, cortisol, RAGE, C/EBP, and CHOP levels were compared at 24-h intervals according to the glucose concentration for up to 72 h. Transfection was confirmed by reduction in the amount of 11β-HSD1 (a). Cortisol levels in hyperglycemic conditions were significantly lower than those in normoglycemic conditions at all time points (b). Significant relationships between hyperglycemic and normoglycemic conditions or negative control (hyperglycemic condition) are denoted by an asterisk, while other correlations are detailed in supplemental materials (b). The RAGE and CHOP mRNA expression increased rapidly at 24 h and decreased after 48 h in hyperglycemic conditions,

but the change was weak under normoglycemic conditions (c, e). The relative mRNA expression levels of RAGE and CHOP at 48- and 72-hour data were separately extracted and presented in additional graphs. (d, f). The mRNA expression of C/EBP was high at 24 h, decreased at 48 h, and increased at 72 h in hyperglycemic conditions. The expression pattern was not observed under normoglycemic conditions (g, h). GAPDH was used as an internal control (c–h). Bars indicate the mean ± SE (N=3; *$p<0.05$, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparison). Negative control; scrambled siRNA control

3. Increase in ER stress under hyperglycemic conditions within the first 24 h was not significantly suppressed by 11β-HSD1 inhibitor treatment, but showed a tendency to alleviate the elevation

Before the experiment, we found that the 11β-HSD1 inhibitor itself could affect cell viability. Absolute toxicity was observed when it exceeded a certain concentration (Fig. 3). To specifically evaluate the relationship of 11β-HSD1 with RAGE, CHOP, and C/EBP observed in the 11β-HSD knockdown condition, we treated NHKs under hyperglycemic conditions with 0.01 μM 11β-HSD1 inhibitor.

Although significant temporal changes in RAGE, CHOP, and C/EBP were inconsistently observed with or without 11β-HSD1 inhibitor treatment, the expression of three markers tended to increase over time under hyperglycemic conditions within 24 h. Despite the absence of prominent differences between the treated and untreated NHKs, the increase in RAGE, CHOP, and C/EBP over time showed a tendency to be weakened in NHKs treated with 11β-HSD1 inhibitor (Fig. 4).

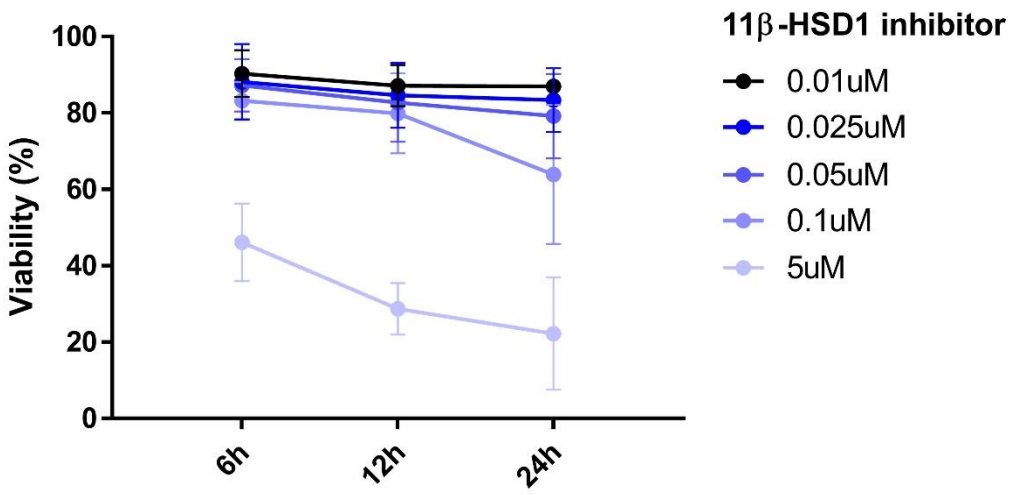

Figure 3. The effect of the 11β-HSD1 inhibitor on cell viability varied depending on its concentration

In the MTT assay, a decrease in cell viability according to the concentration of the 11β-HSD1 inhibitor was definitive. To examine the efficacy of 11β-HSD1 inhibitor treatment on cell culture, it was necessary to select a concentration with little effect on cell viability.

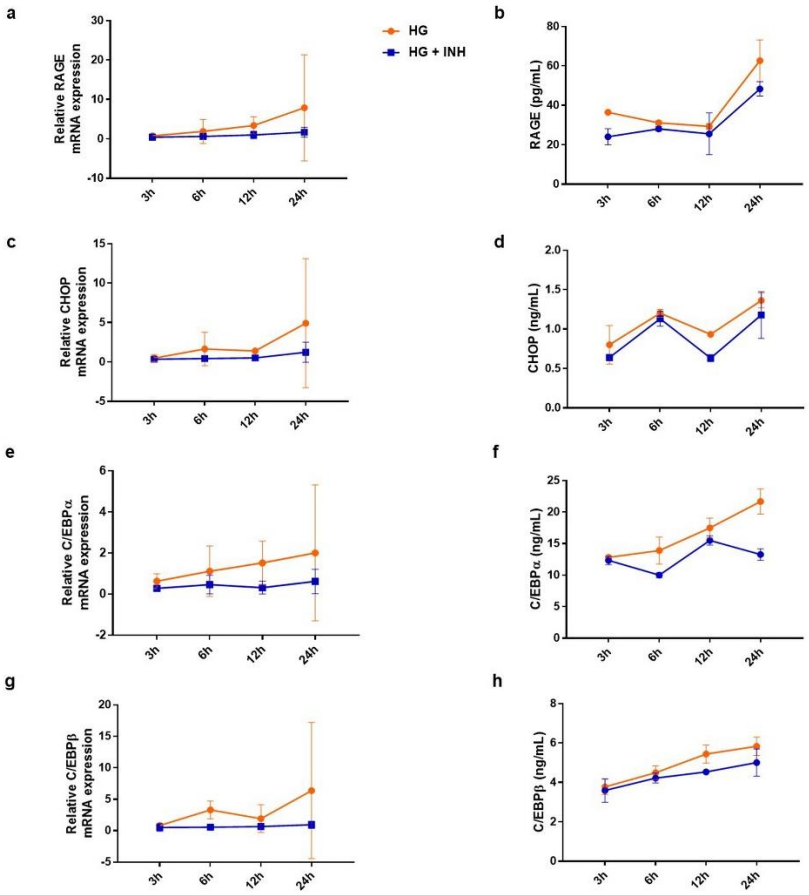

Figure 4. Increasing the expression of RAGE, CHOP, and C/EBP up to the first 24 h under the hyperglycemic condition was partially suppressed by the 11β-HSD1 inhibitor

Changes in mRNA expression and protein levels were observed at 3-h intervals up to 24 h after treatment with 11β-HSD1 inhibitor in normal human keratinocytes under hyperglycemic conditions. The mRNA expressions and protein levels of RAGE and CHOP in hyperglycemic conditions showed a tendency to increase with time but did not show a statistically significant difference compared to the 11β-HSD1 inhibitor-treated condition (a–d). The mRNA expressions

and protein levels of C/EBPα and C/EBPβ showed a gradual increase in hyperglycemic conditions over time, but these changes were insignificant in inhibitor-treated conditions (e–h). GAPDH was used as an internal control (a, c, e, g). The dots with error bars indicate the mean ± SE (N=3). HG; hyperglycemia, HG + INH; hyperglycemic condition treated with an 11β-HSD1 inhibitor.

4. Inhibition of ER stress effected down-regulation of 11β-HSD1 and cortisol under hyperglycemic condition

To investigate the impact of ER stress on 11β-HSD1 expression, changes in cortisol and 11β-HSD1 levels in NHKs were examined after treatment with 2 μM 4-PBA, an ER stress inhibitor, under hyperglycemic conditions (Fig. 5). Time-dependent increase patterns of both 11β-HSD1 and cortisol levels were not observed in NHKs when treated with 4-PBA under hyperglycemic conditions (Fig. 6). In particular, 11β-HSD1 and cortisol levels of hyperglycemic conditions treated with 4-PBA were significantly lower than those of untreated after 48 hours.

In addition, we examined the changes after treatment with thapsigargin (TG), an ER stress activator, in NHKs under normoglycemic conditions (Fig. 7). TG-treated NHKs showed increasing CHOP levels with increasing concentrations of TG. After treatment with TG, the concentration of 11β-HSD1 was also higher than that in the control. However, a quantitative increase in 11β-HSD1 expression according to the concentration of TG was not observed. Therefore, ER stress plays a pivotal role in inducing changes in 11β-HSD1 and cortisol.

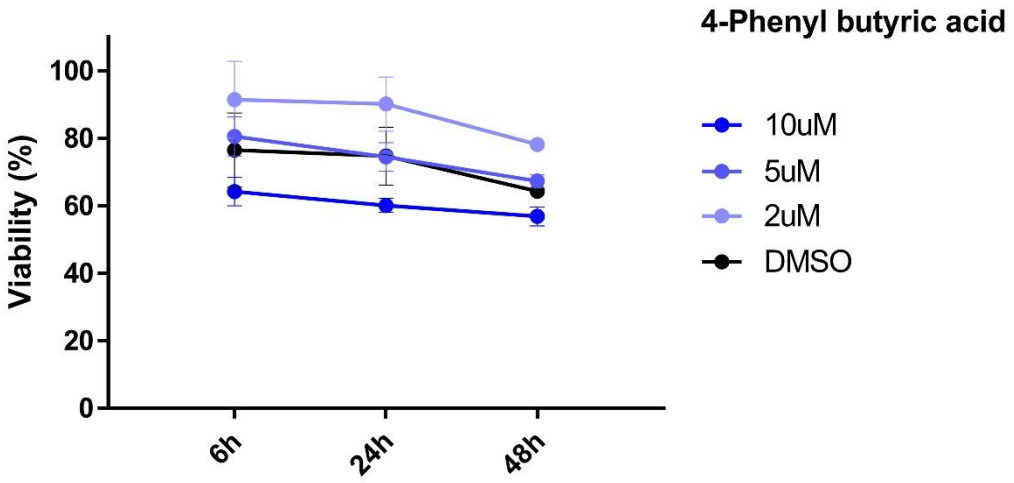

Figure 5. The effect of 4-Phenyl butyric acid (ER stress inhibitor) on cell viability varied depending on its concentration

The MTT assay for 4-Phenyl butyric acid (4-PBA) showed that cell viability decreased in normal human keratinocyte culture as the concentration of 4-PBA increased.

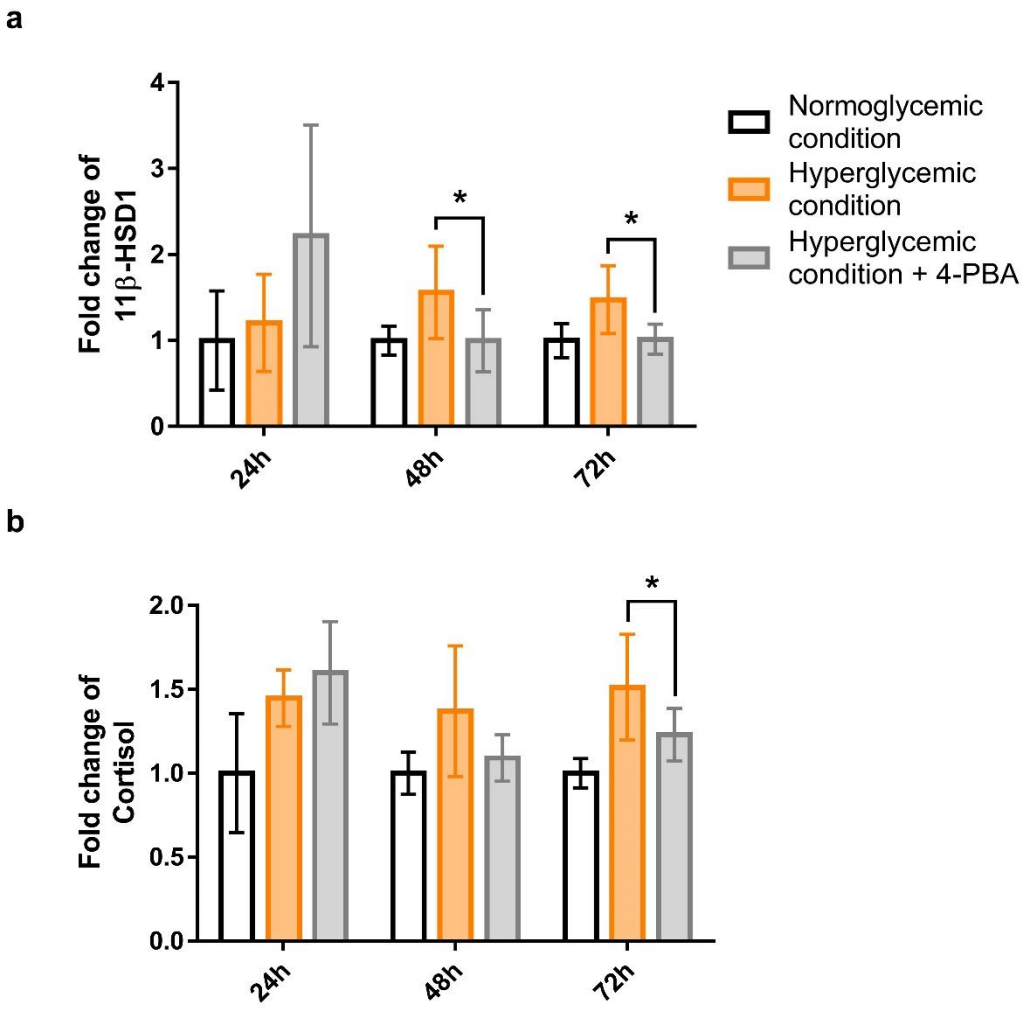

Figure 6. The inhibition of ER stress suppressed the secretion of 11β-HSD1 and cortisol stimulated by hyperglycemia

Normal human keratinocytes (NHKs) under hyperglycemic condition were treated with 4-Phenyl butyric acid (4-PBA), an ER stress inhibitor. When the value under normoglycemic conditions was 1, the change in each condition was expressed as a “fold change”. In NHK treated with 4-PBA,

under hyperglycemic conditions, the level of 11β-HSD1 was significantly lower than that of the untreated NHKs under the same hyperglycemic condition after 48 hours (a). After 72 hours, the level of cortisol was also significantly lower in 4-PBA treated NHKs than in untreated NHKs (b). Significant relationships between hyperglycemic condition and hyperglycemic condition treated with 4-PBA are denoted by an asterisk. Further detailed post-hoc analysis results can be found in the supplemental material. GAPDH was used as an internal control (a, b). Bars indicate the mean ± SE (N=8; *$p<0.05$, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparison).

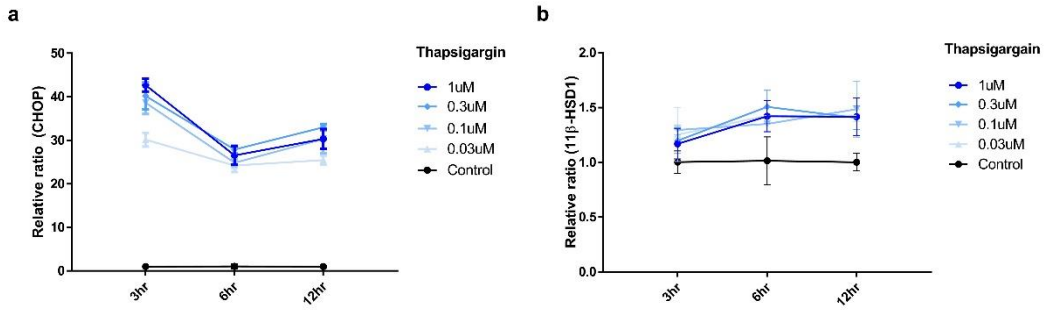

Figure 7. Treatment with an ER stress activator in cell culture resulted in increased levels of 11β-HSD1

For the normoglycemic conditions, normal human keratinocytes were treated with thapsigargin (TG). A proportional increase in CHOP was observed as the concentration of TG increased. In addition, the 11β-HSD1 group showed an increased tendency after TG treatment compared to the controls.

5. Hyperglycemic condition, high ER stress state, increased keratinocyte differentiation in short period, at least 72 hours

Changes in keratinocyte differentiation markers including filaggrin, loricrin, and involucrin were evaluated in NHKs at different glucose concentrations. From 24 hours onward, the mRNA levels of filaggrin, loricrin, and involucrin in NHKs under hyperglycemic condition were significantly higher than those in NHKs under normoglycemic condition (Fig. 8). This elevation was maintained for up to 72 hours. In NHKs under hyperglycemic condition treated with 4-PBA, all three marker levels were significantly lower than those under hyperglycemic condition without 4-PBA treatment.

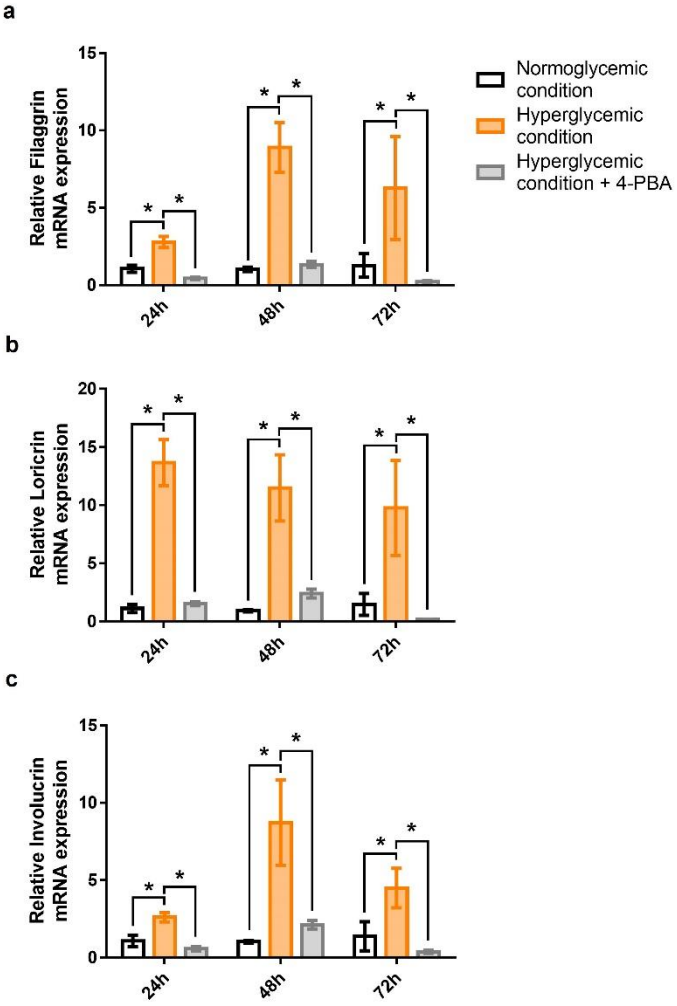

Figure 8. Hyperglycemia increased keratinocyte differentiation, the effect of which was reduced by inhibition of ER stress

The mRNA level of filaggrin in normal human keratinocytes (NHKs) under hyperglycemic condition was significantly higher than that under normoglycemic conditions at all time points (a). The mRNA levels of loricrin and involucrin were significantly higher than those observed in NHKs under normoglycemic condition (b, c). In NHKs under hyperglycemic condition treated

with 4-PBA, all three parameters were significantly decreased compared to the untreated NHKs. GAPDH was used as an internal control (a–c). Bars indicate the mean ± SE (N=5; *p<0.05, Student's t-test). 4-PBA; 4-Phenyl butyric acid

6. Fourteen-week-old db/db mice showed different hormonal profile compared to 8-week-old db/db mice

The 14-week-old db/db mice showed higher serum glucose level, weight, and serum AGE than the controls, suggesting that the setting of db/db mice with DM condition was appropriate (Fig. 9). Serum corticosterone and ACTH levels in 14-week-old db/db mice were significantly higher than those in 8-week-old db/db mice (Fig. 10a, 10b). It seems that hyperglycemia induces the activation of HPA axis.

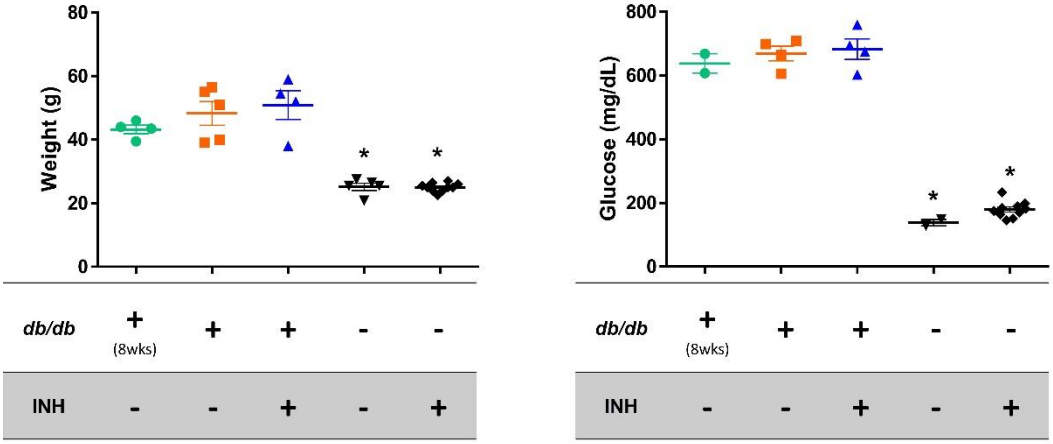

Figure 9. The db/db mice exhibited distinct clinical features

The db/db mice showed significantly higher weight and glucose concentrations than the control mice. *p<0.05, compared to the value of 14-week-old db/db mice treated with the vehicle

7. Elevated 11β-HSD1 and corticosterone were observed in 14-week-old db/db mice, similar to the in vitro results

Compared to 8-week-old db/db mice, 14-week-old db/db mice showed relatively higher serum AGE, skin 11β-HSD1, and stratum corneum (SC) corticosterone (Fig. 10c–10e). However, there was no significant difference in CHOP and C/EBP levels between the two groups (Fig. 10f–10h). In terms of skin barrier function, a significant decrease in SC integrity was observed in 14-week-old db/db mice compared to that in 8-week-old db/db mice (Fig. 11e). Basal transepidermal water loss (TEWL) and SC pH tended to increase in 14-week-old db/db mice (Fig. 11a, 11b). These results suggest that the long-standing condition of DM acts as a precursor to 11β-HSD1 and cortisol elevation, and consequently may induce abnormal skin barrier function at the mouse level.

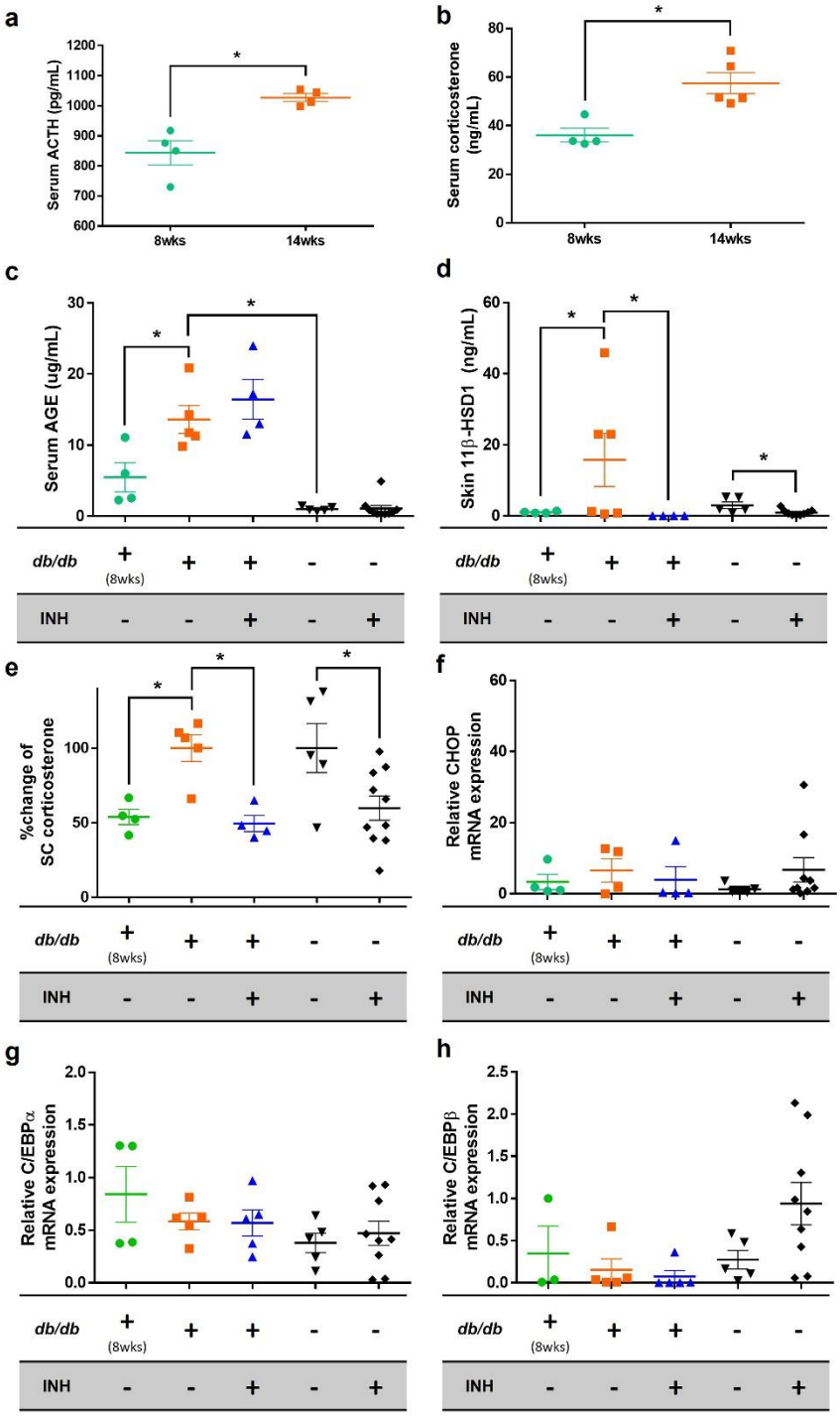

Figure 10. Serum levels of ACTH, corticosterone, and AGE and skin levels of 11β-HSD1 and corticosterone were increased in 14-week-old db/db mice compared to 8-week. Skin 11β-HSD1 and corticosterone production were inhibited by a topical 11β-HSD1 inhibitor

Serum ACTH and corticosterone levels were compared between 8-week-old and 14-week-old db/db mice (N = 5 per group; *p<0.05, Student's t-test) (a, b). Serum AGE, skin 11β-HSD1, SC corticosterone, skin CHOP mRNA expression, and skin C/EBP mRNA expression levels were compared 2 weeks after topical application of vehicle and 11β-HSD1 inhibitor in db/db mice and controls (c–h). When the average corticosterone level of mice untreated with topical 11β-HSD1 inhibitor within each strain was 100, the amount of change in each group was expressed as "% change of SC corticosterone" (e). GAPDH was used as an internal control (f–h). The scatter plot with error bars indicates the mean ± SE (N = 5 per group, except for the group with controls treated with 11β-HSD1 inhibitor (N=10); *p<0.05, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparison). db/db; db/db mice. INH; 11β-HSD1 inhibitor treatment.

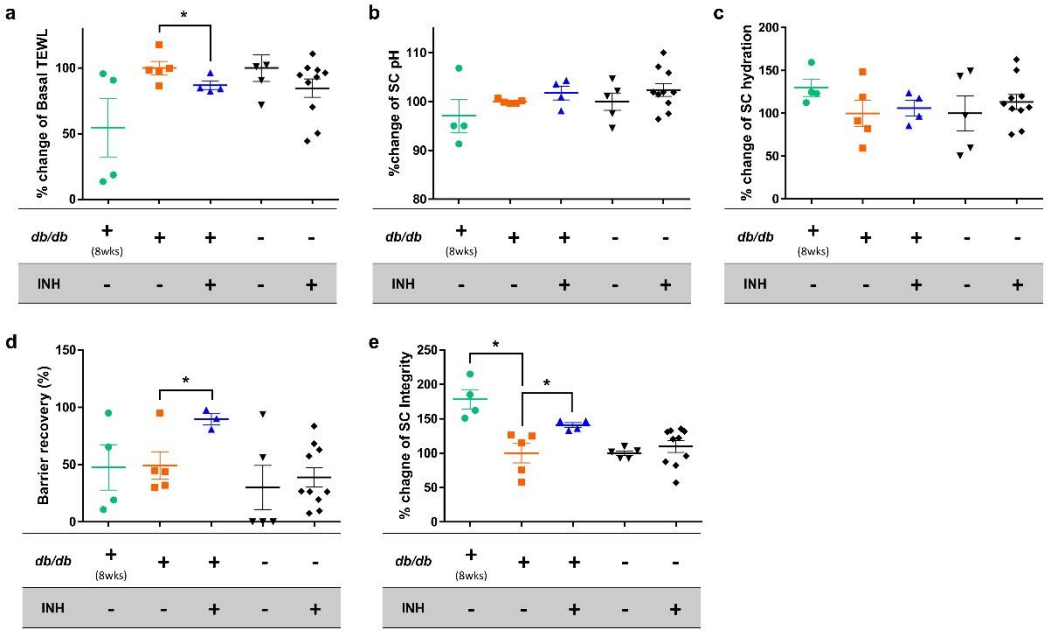

Figure 11. Skin barrier function can be improved by applying the topical 11β-HSD1 inhibitor in db/db mice

Skin barrier function was measured 2 weeks after topical application of the vehicle and 11β-HSD1 inhibitor in db/db mice and controls. Evaluation of skin barrier function included basal TEWL, SC pH, SC hydration, barrier recovery, and SC integrity (a–e). The 14-week-old db/db mice treated with topical 11β-HSD1 inhibitor showed a significantly lower TEWL, higher barrier recovery rate, and higher SC integrity compared to the 14-week-old db/db mice treated with the vehicle (a, d, e). When the average level of mice untreated with topical 11β-HSD1 inhibitor within each strain (db/db and control) was 100, the amount of change in each group was expressed as "% change". The scatter plot with error bars indicates mean ± SE (N = 5 per group, except for the group with controls treated with 11β-HSD1 inhibitor (N=10); *$p<0.05$, one-way ANOVA followed by the

Bonferroni-Dunn test for multiple comparison). db/db; db/db mice. INH; 11β-HSD1 inhibitor treatment.

8. Inhibition of skin corticosterone production and improvement of skin barrier function were observed in 14-week-old db/db mice treated with a topical 11β-HSD1 inhibitor

After applying the vehicle and a topical 11β-HSD1 inhibitor for 2 weeks, protein and mRNA expressions in the skin and skin barrier function were measured. The level of SC corticosterone was lower in 14-week-old db/db mice treated with a topical 11β-HSD1 inhibitor than in 14-week-old db/db mice treated with the vehicle. Even in the control group, mice treated with the topical 11β-HSD1 inhibitor showed lower levels of SC corticosterone than vehicle-treated mice.

No significant difference in epidermal C/EBP was observed between the topical 11β-HSD1 inhibitor-treated and vehicle-treated 14-week-old db/db mice (Fig. 10g, 10h). Notably, the CHOP level of 14-week-old db/db mice treated with the 11β-HSD1 inhibitor showed a decreasing tendency, although not significant, compared to that of vehicle-treated mice (Fig. 10f).

The 14-week-old db/db mice treated with topical 11β-HSD1 inhibitor showed a lower basal TEWL, higher barrier recovery rate, and higher SC integrity than the vehicle-treated mice (Fig. 11a, 11d, 11e), although skin pH and SC hydration were not significantly different (Fig. 11b, 11c). Accordingly, it is suggested that inhibition of skin 11β-HSD1 significantly improves epidermal defects by alleviating local GC excess.

Discussion

In vivo and in vitro studies have shown that hyperglycemic conditions cause structural and functional defects in the epidermis in both non-wounded and wounded skin[13,15,24,25]. However, these findings are mainly based on phenomenological observations, and the specific mechanisms regarding the causal relationship between hyperglycemia and impaired skin barrier function remain mostly unknown. Although various signaling pathways are presumed to be involved in the process by which high glucose conditions induce metabolic abnormalities in endocrinological fields, studies focusing on keratinocytes are limited.

HPA axis activation and elevation of systemic GC induced by hyperglycemic conditions were observed in this study. Although it is debatable whether the metabolic syndrome characterized by insulin resistance elevates systemic cortisol levels clinically, there is at least a consistency in the HPA axis abnormalities including aberrant cortisol response to external stress[26-29]. Thus, it is speculated that DM drives the systemic balance of GC in a steroidogenic (hypercortisolemia) manner.

The identification of key enzymes involved in steroidogenesis, which converts cholesterol into steroid hormones, and the understanding of the subsequent signaling pathways leading to GC production in the skin, akin to those observed in classic steroidogenic tissues like gonads and adrenal glands, support the notion of the skin's physiological role and independent status as a peripheral neuroendocrine organ[2,3]. We previously reported that activating the HPA axis by systemic stimuli, such as psychological stress, could affect local GC activation[30]. Furthermore, it is thought that the skin has its own cycle corresponding to the systemic HPA axis or interactive

crosstalk[31-35]. Accordingly, systemic GC excess in the background of DM may lead to a mainstream stimulation of local GC production (higher secretion of cortisol in keratinocytes).

The qualitative and quantitative increase in 11β-HSD activity, which enhances the local availability of GC, attenuates the acute inflammation of keratinocytes[36-38]. The regulation of 11β-HSD1 has been shown to be influenced by various environmental stimuli, contributing to the maintenance of epidermal barrier homeostasis. For instance, studies have demonstrated the activation of 11β-HSD1 in response to UV exposure in human skin ex-vivo[39]. Additionally, locally dysfunctional steroidogenic pathways have been clinically observed in chronic inflammatory dermatosis, such as atopic dermatitis and psoriasis[38,40]. It has been demonstrated in aged mice that chronically activated 11β-HSD1 plays an important role in age-related epidermal dysfunction and elevation of cortisol[7,41]. Considering that the epidermal alteration in DM corresponds to boosted aging skin[13,15], 11β-HSD1 activation in the setting of DM shown in this study may superimpose the acceleration of local GC excess. Therefore, it can be assumed that 11β-HSD1 induced GC elevation disrupts GC homeostasis and leads to gradual GC excess in local steroidogenesis of DM, in addition to mainstream GC excess driven by HPA axis activation.

In contrast to the relatively well-established association between ER stress and hyperglycemia, studies on the relationship between ER stress and 11β-HSD1 in the skin are limited. Based on the consistent changes in 11β-HSD1 and cortisol levels in NHKs by regulation of ER stress in this study, hyperglycemia-triggered high ER stress mediates a mainstream process including 11β-HSD1-mediated cortisol production. Furthermore, an increased keratinocyte differentiation, induced by high ER stress, is in line with the findings of previous studies of pathological keratinization disorders with aberrant ER stress[42-44].

However, aside from the regulation of 11β-HSD1 by ER stress, 11β-HSD1-inhibition suppressed ER stress to some extent in this study. This suggests that 11β-HSD1-mediated cortisol production is not a sub-signaling step unilaterally governed by ER stress. In terms of the possibility of a link between ER stress and GC (or 11β-HSD1), the following possibilities can be considered.

There are conflicting results regarding the relationship between GC and ER stress[45,46]. GC was also reported to increase ER stress under certain conditions showing tissue-specificity such as in trabecular meshwork cells in GC-induced glaucoma, in hippocampus with overexposure of endogenous GC during chronic stress, and in endotheliocytes with GC-induced osteonecrosis of femoral head[47-49]. GC was found to activate certain signaling pathways, including the IRE1α/XBP-1ER and autophagy[50,51]. This suggests functional complexity between ER stress and the GC signaling pathway. Hence, it seems that inhibition of 11β-HSD1 weakened additional GC elevation and eventually led to a decrease in ER stress in the study.

Altogether, hyperglycemia in DM induces high ER stress and a systemic GC surge through HPA axis activation, which is the cornerstone of local GC elevation. An increase in ER stress drives 11β-HSD1-mediated GC production which exacerbates local GC excess. ER stress is bidirectionally influenced by the GC signaling pathway. Local GC excess including increased GC by 11β-HSD1 activation causes increased epidermal differentiation. However, prolonged GC excess leads to the suppression of keratinocyte proliferation which has a detrimental effect on the epidermis, resulting in skin barrier dysfunction[15,52].

Nevertheless, several points in this study need to be interpreted carefully. First, the decrease in viability following the prolonged cell-breeding period can cause an increase in ER

stress through a complex mechanism related to unintentional cell apoptosis. Consequently, accurate quantitative evaluation of the pure increment of ER stress due to hyperglycemia is limited. Regardless of the presence or absence of the skin HPA axis, among the production of systemic GC excess-driven local GC, it is difficult to accurately estimate the proportion of GC that 11β-HSD1 is responsible for. Conversely, there is a limit to quantifying the effect of local GC production on the systemic HPA axis via or non-via 11β-HSD1. Another limitation of the in vitro study is that while the temporal pattern of changes was relatively well-defined in the 11β-HSD1 siRNA transfection experiment, the levels of 11β-HSD1 in siRNA-transfected condition did not show a significant difference compared to the scrambled siRNA control. This suggests incomplete knockdown of 11β-HSD1, warranting cautious interpretation of the results. However, because body weight, glucose, and serum AGE did not change in the murine study even after 2 weeks of topical 11β-HSD1 inhibitor application, it is quite clear that inhibition of skin 11β-HSD1 is a sufficient tool to control local GC excess without significant systemic changes. Further studies are required to determine whether the findings of this study can be similarly observed under other diabetes-related conditions that may induce elevated ER stress, such as insulin resistance, obesity, and metabolic syndrome.

In conclusion, DM could directly accelerate the impairment of skin barrier function with an increase in ER stress. Furthermore, DM is thought to induce systemic and local GC synergy, accompanied by higher local GC excess due to the action of 11β-HSD1. Thus, the clinical efficacy for the improvement of skin barrier dysfunction in DM through the inhibition of 11β-HSD1 is expected.

REFERENCES

1. Slominski, A. T. et al. Neuroendocrine signaling in the skin with a special focus on the epidermal neuropeptides. Am J Physiol Cell Physiol **323**, C1757–c1776 (2022).
2. Slominski, A. T., Zmijewski, M. A., Plonka, P. M., Szaflarski, J. P. & Paus, R. How UV Light Touches the Brain and Endocrine System Through Skin, and Why. Endocrinology **159**, 1992–2007 (2018).
3. Slominski, R. M. et al. The significance of CYP11A1 expression in skin physiology and pathology. Mol Cell Endocrinol **530**, 111238 (2021).
4. Slominski, A. et al. Steroidogenesis in the skin: implications for local immune functions. J Steroid Biochem Mol Biol **137**, 107–123 (2013).
5. Terao, M. & Katayama, I. Local cortisol/corticosterone activation in skin physiology and pathology. J Dermatol Sci **84**, 11–16 (2016).
6. Kao, J. S. et al. Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of epidermal lipid synthesis accounts for functional abnormalities. J Invest Dermatol **120**, 456–464 (2003).
7. Tiganescu, A. et al. 11β-Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. J Clin Invest **123**, 3051–3060 (2013).
8. Choi, E. H. Aging of the skin barrier. Clin Dermatol **37**, 336–345 (2019).
9. Tiganescu, A., Walker, E. A., Hardy, R. S., Mayes, A. E. & Stewart, P. M. Localization, age- and site-dependent expression, and regulation of 11β-hydroxysteroid dehydrogenase type 1 in skin. J Invest Dermatol **131**, 30–36 (2011).
10. Boudon, S. M. et al. Novel 11β-hydroxysteroid dehydrogenase 1 inhibitors reduce

cortisol levels in keratinocytes and improve dermal collagen content in human ex vivo skin after exposure to cortisone and UV. PLoS One **12**, e0171079 (2017).

11. Terao, M. et al. 11β-Hydroxysteroid dehydrogenase-1 is a novel regulator of skin homeostasis and a candidate target for promoting tissue repair. PLoS One **6**, e25039 (2011).
12. Terao, M. et al. 11β-hydroxysteroid dehydrogenase 1 specific inhibitor increased dermal collagen content and promotes fibroblast proliferation. PLoS One **9**, e93051 (2014).
13. Park, H. Y. et al. A long-standing hyperglycaemic condition impairs skin barrier by accelerating skin ageing process. Exp Dermatol **20**, 969–974 (2011).
14. Karadag, A. S., Ozlu, E. & Lavery, M. J. Cutaneous manifestations of diabetes mellitus and the metabolic syndrome. Clin Dermatol **36**, 89–93 (2018).
15. Kim, J. H. et al. Impaired permeability and antimicrobial barriers in type 2 diabetes skin are linked to increased serum levels of advanced glycation end-product. Exp Dermatol **27**, 815–823 (2018).
16. Seo, S. H., Kim, S. E. & Lee, S. E. ER stress induced by ER calcium depletion and UVB irradiation regulates tight junction barrier integrity in human keratinocytes. J Dermatol Sci **98**, 41–49 (2020).
17. Sano, R. & Reed, J. C. ER stress-induced cell death mechanisms. Biochim Biophys Acta **1833**, 3460–3470 (2013).
18. Ozcan, U. et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science **313**, 1137–1140 (2006).
19. Piperi, C., Adamopoulos, C., Dalagiorgou, G., Diamanti-Kandarakis, E. & Papavassiliou, A. G. Crosstalk between advanced glycation and endoplasmic reticulum stress: emerging therapeutic targeting for metabolic diseases. J Clin Endocrinol Metab **97**, 2231–2242

(2012).

20. Burgos-Morón, E. et al. Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. J Clin Med. **8**, 1385 (2019).
21. Khan, S., Wang, C. H. ER stress in adipocytes and insulin resistance: mechanisms and significance (Review). Mol Med Rep **10,** 2234-2240 (2014).
22. Kim, J. et al. Cadmium-induced ER stress and inflammation are mediated through C/EBP-DDIT3 signaling in human bronchial epithelial cells. Exp Mol Med **49**, e372 (2017).
23. Pan, H. C. et al. Reciprocal modulation of C/EBP-α and C/EBP-β by IL-13 in activated microglia prevents neuronal death. Eur J Immunol **43**, 2854–2865 (2013).
24. Okano, J. et al. Hyperglycemia Induces Skin Barrier Dysfunctions with Impairment of Epidermal Integrity in Non-Wounded Skin of Type 1 Diabetic Mice. PLoS One **11**, e0166215 (2016).
25. Kim, M. et al. Enrichment of Short-Chain Ceramides and Free Fatty Acids in the Skin Epidermis, Liver, and Kidneys of db/db Mice, a Type 2 Diabetes Mellitus Model. Biomol Ther (Seoul) **27**, 457–465 (2019).
26. Weaver, J. U., Kopelman, P. G., McLoughlin, L., Forsling, M. L. & Grossman, A. Hyperactivity of the hypothalamo-pituitary-adrenal axis in obesity: a study of ACTH, AVP, beta-lipotrophin and cortisol responses to insulin-induced hypoglycaemia. Clin Endocrinol (Oxf) **39**, 345–350 (1993).
27. Misra, M. et al. Lower growth hormone and higher cortisol are associated with greater visceral adiposity, intramyocellular lipids, and insulin resistance in overweight girls. Am J Physiol Endocrinol Metab **295**, E385–392 (2008).
28. Reinehr, T. et al. Relationships between 24-hour urinary free cortisol concentrations and

metabolic syndrome in obese children. J Clin Endocrinol Metab **99**, 2391–2399 (2014).

29. Hackett, R. A., Kivimäki, M., Kumari, M. & Steptoe, A. Diurnal Cortisol Patterns, Future Diabetes, and Impaired Glucose Metabolism in the Whitehall II Cohort Study. J Clin Endocrinol Metab **101**, 619–625 (2016).
30. Choe, S. J. et al. Psychological Stress Deteriorates Skin Barrier Function by Activating 11β-Hydroxysteroid Dehydrogenase 1 and the HPA Axis. Sci Rep **8**, 6334 (2018).
31. Thiboutot, D. et al. Human skin is a steroidogenic tissue: steroidogenic enzymes and cofactors are expressed in epidermis, normal sebocytes, and an immortalized sebocyte cell line (SEB-1). J Invest Dermatol **120**, 905–914 (2003).
32. Ito, N. et al. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. Faseb j **19**, 1332–1334 (2005).
33. Inui, S. & Itami, S. Androgen actions on the human hair follicle: perspectives. Exp Dermatol **22**, 168–171 (2013).
34. Nikolakis, G. & Zouboulis, C. C. Skin and glucocorticoids: effects of local skin glucocorticoid impairment on skin homeostasis. Exp Dermatol **23**, 807–808 (2014).
35. Wierzbicka, J. M., Żmijewski, M. A., Antoniewicz, J., Sobjanek, M. & Slominski, A. T. Differentiation of Keratinocytes Modulates Skin HPA Analog. J Cell Physiol **232**, 154–166 (2017).
36. Itoi, S., Terao, M., Murota, H. & Katayama, I. 11β-Hydroxysteroid dehydrogenase 1 contributes to the pro-inflammatory response of keratinocytes. Biochem Biophys Res Commun **440**, 265–270 (2013).
37. Terao, M. et al. Local Glucocorticoid Activation by 11β-Hydroxysteroid Dehydrogenase 1 in Keratinocytes: The Role in Hapten-Induced Dermatitis. Am J Pathol **186**, 1499–1510 (2016).

38. Lee, N. R. et al. Role of 11β-hydroxysteroid dehydrogenase type 1 in the development of atopic dermatitis. Sci Rep **10**, 20237 (2020).

39. Skobowiat, C., Sayre, R. M., Dowdy, J. C. & Slominski, A. T. Ultraviolet radiation regulates cortisol activity in a waveband-dependent manner in human skin ex vivo. Br J Dermatol **168**, 595–601 (2013).

40. Slominski, A. T., Brożyna, A. A. & Tuckey, R. C. Cutaneous Glucocorticoidogenesis and Cortisol Signaling Are Defective in Psoriasis. J Invest Dermatol **137**, 1609–1611 (2017).

41. Kim, B. J. et al. Increased Expression of 11β-Hydroxysteroid Dehydrogenase Type 1 Contributes to Epidermal Permeability Barrier Dysfunction in Aged Skin. Int J Mol Sci **22**, 5750 (2021).

42. Mahanty, S. et al. Keratinocyte differentiation promotes ER stress-dependent lysosome biogenesis. Cell Death Dis **10**, 269 (2019).

43. Sugiura, K. Unfolded protein response in keratinocytes: impact on normal and abnormal keratinization. J Dermatol Sci **69**, 181–186 (2013).

44. Celli, A. et al. Endoplasmic reticulum Ca2+ depletion activates XBP1 and controls terminal differentiation in keratinocytes and epidermis. Br J Dermatol **164**, 16–25 (2011).

45. Das, I. et al. Glucocorticoids alleviate intestinal ER stress by enhancing protein folding and degradation of misfolded proteins. J Exp Med **210**, 1201–1216 (2013).

46. Fujii, Y. et al. The effect of dexamethasone on defective nephrin transport caused by ER stress: a potential mechanism for the therapeutic action of glucocorticoids in the acquired glomerular diseases. Kidney Int **69**, 1350–1359 (2006).

47. Bao, A. M., Meynen, G. & Swaab, D. F. The stress system in depression and neurodegeneration: focus on the human hypothalamus. Brain Res Rev **57**, 531–553 (2008).

48. Gao, Y., Zhu, H., Wang, Q., Feng, Y. & Zhang, C. Inhibition of PERK Signaling Prevents Against Glucocorticoid-induced Endotheliocyte Apoptosis and Osteonecrosis of the Femoral Head. Int J Biol Sci **16**, 543–552 (2020).

49. Kasetti, R. B., Maddineni, P., Millar, J. C., Clark, A. F. & Zode, G. S. Increased synthesis and deposition of extracellular matrix proteins leads to endoplasmic reticulum stress in the trabecular meshwork. Sci Rep **7**, 14951 (2017).

50. Gao, Y. et al. Glucocorticoid-activated IRE1α/XBP-1s signaling: an autophagy-associated protective pathway against endotheliocyte damage. Am J Physiol Cell Physiol **315**, C300–c309 (2018).

51. Mihailidou, C., Panagiotou, C., Kiaris, H., Kassi, E. & Moutsatsou, P. Crosstalk between C/EBP homologous protein (CHOP) and glucocorticoid receptor in lung cancer. Mol Cell Endocrinol **436**, 211–223 (2016).

52. Spravchikov, N. et al. Glucose effects on skin keratinocytes: implications for diabetes skin complications. Diabetes **50**, 1627–1635 (2001).

53. Lan, C. C. et al. High-Glucose Environment Inhibits p38MAPK Signaling and Reduces Human β-Defensin-3 Expression [corrected] in Keratinocytes. Mol Med **17**, 771–779 (2011).

54. Gkogkolou, P. et al. Protection of glucotoxicity by a tripeptide derivative of α-melanocyte-stimulating hormone in human epidermal keratinocytes. Br J Dermatol **180**, 836–848 (2019).

55. Huang, S. M. et al. High glucose environment induces M1 macrophage polarization that impairs keratinocyte migration via TNF-α: An important mechanism to delay the diabetic wound healing. J Dermatol Sci **96**, 159–167 (2019).

56. Oster, H. et al. The circadian rhythm of glucocorticoids is regulated by a gating

mechanism residing in the adrenal cortical clock. Cell Metab **4**, 163–173 (2006).

57. Gong, S. et al. Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice. PLoS One **10**, e0117503 (2015).

국 문 초 록

고혈당으로 활성화된
11β-hydroxysteroid dehydrogenase type 1에
의한 소포체 스트레스 증가 및 피부 장벽 기능 이상

이영빈

연세대학교 일반대학원 의학과

연구배경

당뇨병이 있는 환자의 피부는 피부 장벽 기능 이상과 피부 지질 이상을 보이며, 이는 전신 혹은 국소적 당질코르티코이드 과다 및 노화 피부에서 나타나는 특징과 유사하다. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1)는 당질코르티코이드의 항상성 조절에 관여하는데, 11β-HSD1는 비활성 상태의 당질코르티코이드를 활성 상태의 당질코르티코이드로 전환한다. 이에 당뇨라는 고혈당 조건이 당질코르티코이드 항상성에 영향을 미치는지 그리고 고혈당 조건의 각질형성세포에서 11β-HSD1가 국소적 당질코르티코이드 과다 상태에 관여하는지 알아보고자 하였다.

연구방법

각질형성세포 배양실험과 db/db 당뇨 마우스 실험에서 고혈당 조건과 정상 혈당 조건 간 11β-HSD1, 활성 상태의 당질코르티코이드 그리고 소포체 스트레스 수치를

비교하였다.

연구결과

각질형성세포 배양액 내 11β-HSD1와 코티솔은 고혈당 조건에서 시간이 지남에 따라 증가하였다. 11β-HSD1 siRNA 전이된 세포에서는 같은 고혈당 조건이더라도 시간에 따른 코티솔 수치의 증가가 나타나지 않았다. 소포체 스트레스 억제제 처치시 고혈당 조건에서 각질형성세포 배양액의 11β-HSD1와 코티솔 생산이 억제되었다. 11β-HSD1 억제제 처치시 각질형성세포 배양액에서 소포체 스트레스가 감소하였는데, 이는 소포체 스트레스와 11β-HSD1 및 당질코르티코이드 사이의 상호작용적 관계를 시사한다. 14주령 db/db 당뇨 마우스 군은 8주령 db/db 당뇨 마우스 군과 비교하여 각질층의 코르티코스테론과 피부 11β-HSD1 수치가 상대적으로 높았다. 국소 11β-HSD1 억제제를 피부에 도포한 db/db 당뇨 마우스 군은 각질층의 코르티코스테론 수치가 감소하였고 피부 장벽 기능이 개선되었다.

결론

당뇨라는 고혈당 상태가 전신적 당질코르티코이드 항상성에 영향을 미치고 피부 11β-HSD1를 활성화시키며, 국소 당질코르티코이드 과다 상태를 유도함으로써 소포체 스트레스 증가 및 피부 장벽 기능의 악화를 초래한다.

주제어 (Keywords): 고혈당; 11β-hydroxysteroid dehydrogenase type 1; 각질형성세포; 소포체 스트레스; 당질코르티코이드; db/db 당뇨 마우스