Chronic stress weakens the skin barrier function owing to increased cortisol sensitivity through the imbalance in the expression of cortisol-metabolizm

ID 372

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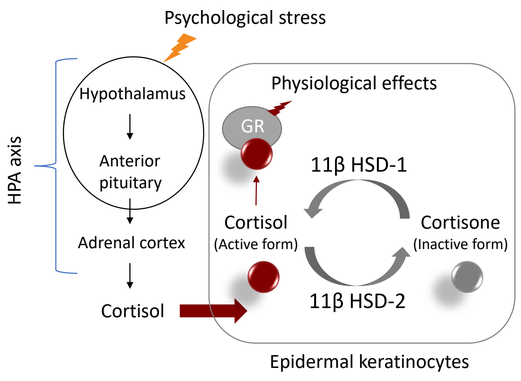
Introduction:

Psychological stress and skin

Cortisol is a hormone released into the bloodstream during physical and psychological stress. It adversely affects the body when maintained at high levels for prolonged durations due to psychological stress. Although a temporary state of high cortisol concentration is caused by physical stress or diurnal variation, skin problems only occur in conditions of chronic high cortisol concentration. Thus, clarifying the effect of high cortisol levels on epidermal keratinocytes is vital to examining the influence of chronic stress conditions on the skin.

Control of intercellular cortisol concentration

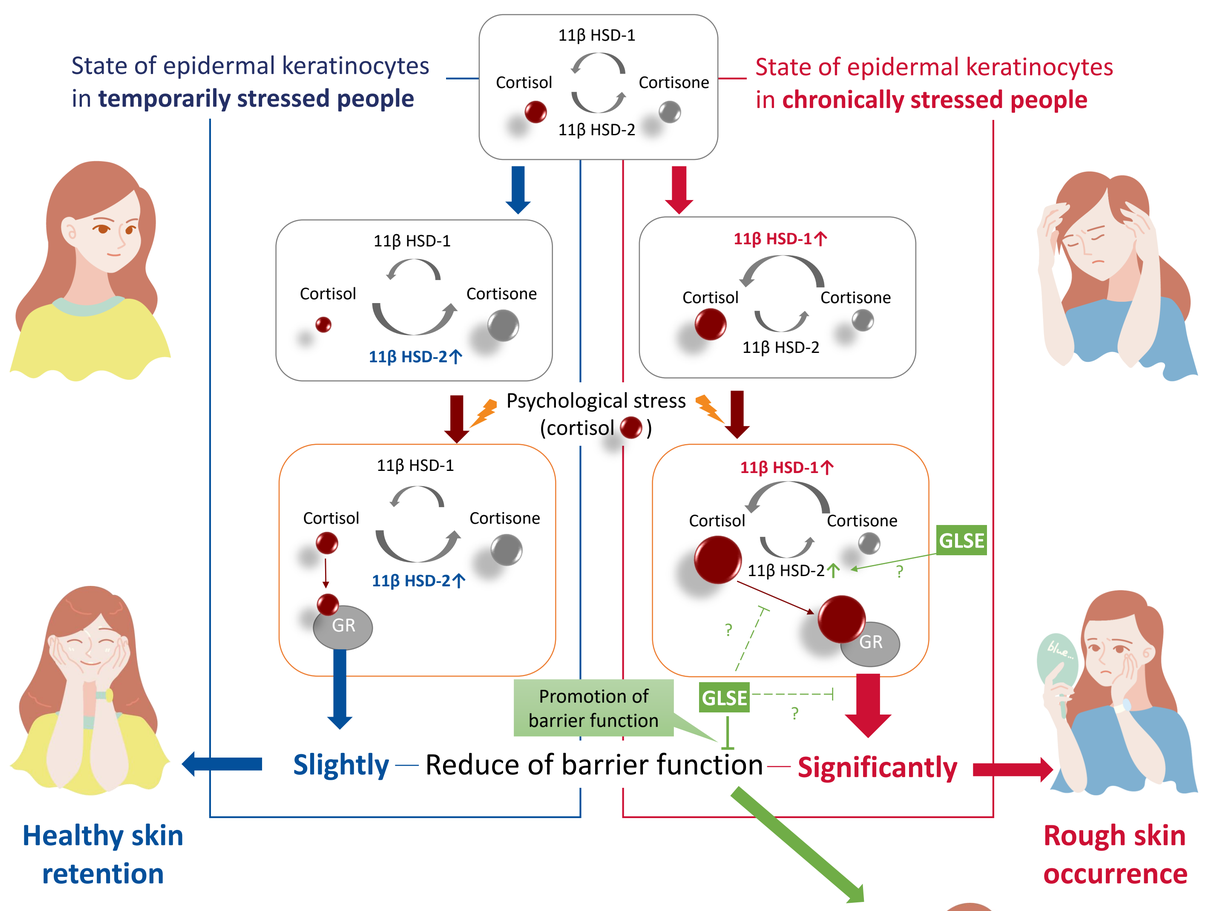
Activated hypothalamic–pituitary–adrenal (HPA) axis is the main regulatory pathway of cortisol synthesis. Cortisol secreted from the adrenal glands is supplied via the blood to various tissues, such as skin. Cortisol exerts various physiological effects by binding to glucocorticoid receptors (GR) in cells. The cortisol-metabolizing enzymes, 11β hydroxysteroid dehydrogenases (11β HSDs), are present in epidermal keratinocytes [1] and regulate intracellular cortisol concentrations. Therefore, cortisol binding to GR is regulated by the cortisol metabolic balance, which depends on the expression levels of 11β HSDs.



Objective

In this study, we investigated the relationship between cortisol exposure and the metabolic capacity of 11β HSDs in keratinocytes and the mechanism responsible for their adverse effects on the skin under chronic stress conditions. Furthermore, we screened for natural extracts that improve skin problems caused by chronic stress.

Conclusions :



The persistence of high cortisol levels due to chronic psychological stress may increase the sensitivity to cortisol by changing the expression levels of 11β HSD-1 and 11β HSD-2, thus enhancing the effect of cortisol on the epidermis and excessively attenuating barrier function. GLSE, which promotes the formation of the barrier function under cortisol in NHEKs, is expected to prevent or ameliorate skin problems caused by chronic stress. Moreover, β-1,3-Glucan was confirmed to be one of active components in GLSE.

Materials & Methods:

Cell culture

Normal human epidermal keratinocytes (NHEKs) were pre-cultured in serum-free keratinocyte growth medium. After that, each cell was cultured with three types of different conditions until differentiation induction.

1. Non-stressed cells; NSCs
NHEKs were untreated with cortisol.

2. Temporarily stressed cells; TSCs
NHEKs were treated with 20 μM cortisol once in three days.

3. Chronically stressed cells; CSCs
NHEKs were treated with 20 μM cortisol daily.

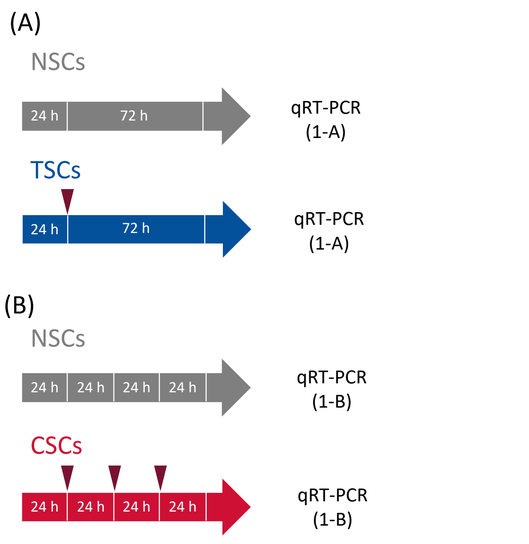
Natural Extract

GLSE
Ganoderma Lucidum Stem Extract
(Ganoderma Lucidum Fruiting Body Extract)

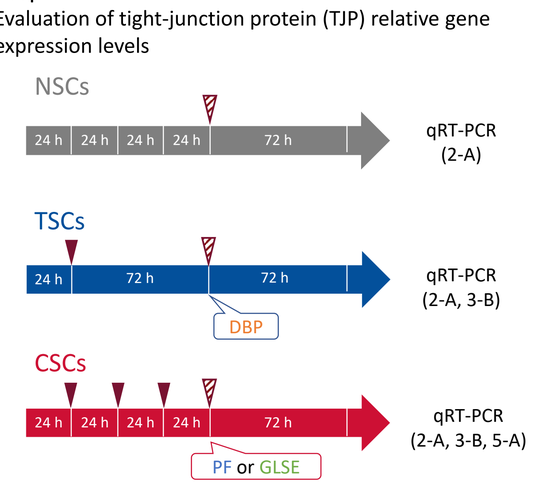
Ganoderma Lucidum, an oriental fungus, is a traditional healthy food with many kinds of nutritious activities, such as insomnia, neurasthenia, inflammation and so on [2]. In China, Ganoderma lucidum is said to be a medicine of immortality. It is reported that Ganoderma Lucidum contained many kinds of bioactive compounds such as polysaccharides (including β-1,3-glucan), triterpenoids, glycopeptides, sterols and so on [3].

Test method

Step Ⅰ
Evaluation of 11β HSD mRNA expression levels



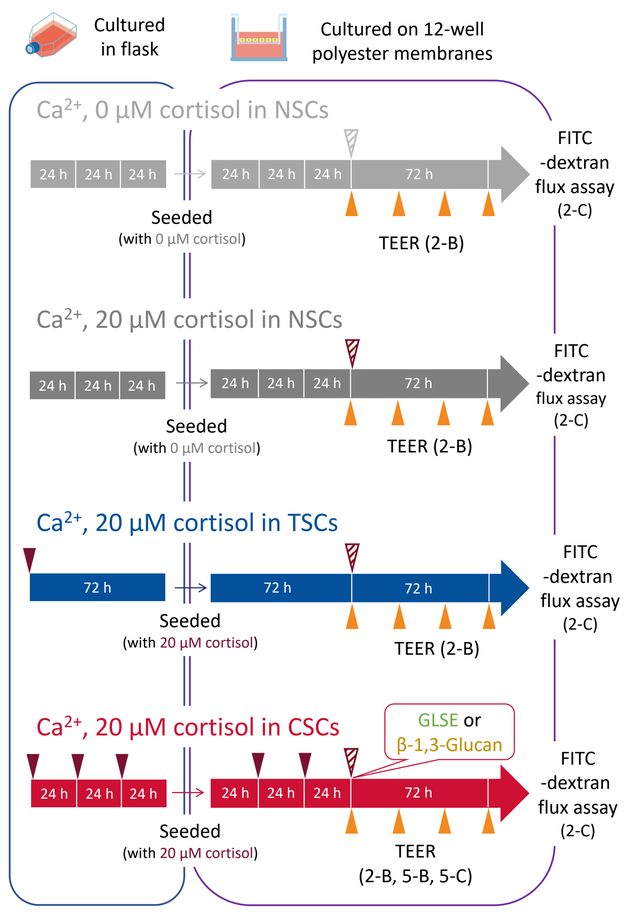
Step Ⅱ
Evaluation of tight-junction protein (TJP) relative gene expression levels



DBP : Dibutyl phthalate (11β HSD-2 inhibitor)
PF : PF915275 (11β HSD-1 inhibitor)

: Add 20 μM cortisol
: Induce differentiation (1.5 mM Ca^{2+}, 0 μM cortisol)
: Induce differentiation (1.5 mM Ca^{2+}, 20 μM cortisol)
: Measure TEER (every 24 h)

Step Ⅲ
Evaluation of barrier function



Results & Discussion:

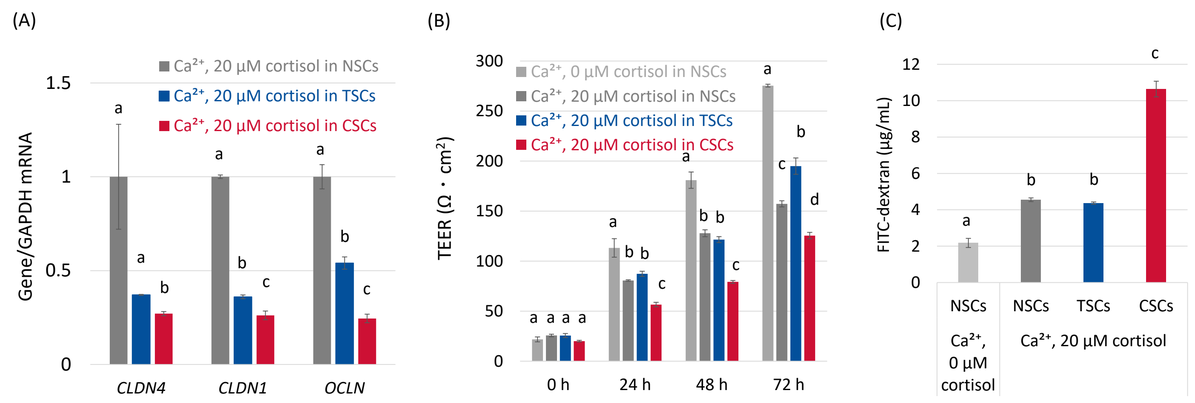
1. 11β HSD mRNA expression levels under different cortisol treatment conditions

11β HSD-1 mRNA expression levels were not different between TSCs and NSCs, whereas 11β HSD-2 mRNA expression levels clearly increased after 72 h of cortisol treatment (A). In contrast, 11β HSD-1 mRNA expression levels in CSCs increased compared to those in NSCs, whereas 11β HSD-2 mRNA expression levels did not (B).

Temporary cortisol treatment reduces the effect of cortisol by inducing 11β HSD-2, whereas continuous cortisol treatment maintains a high concentration of intracellular cortisol by inducing 11β HSD-1 but not 11β HSD-2.

mean ± SEM, n = 3, ** $p < 0.01$, vs. NSCs using t-test.

2. Comparison of barrier function under different cortisol treatment conditions

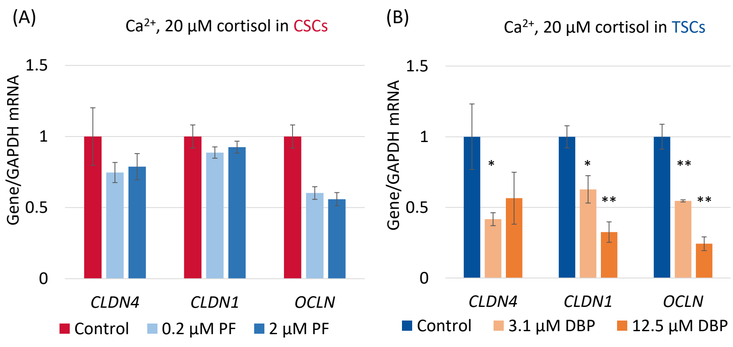


mean ± SEM, n = 3; different letters (a, b, c, or d) indicate significant differences ($p < 0.05$) at each given time point using Tukey's test.

When differentiation was induced under the presence of 20 μM cortisol, the expression levels of *CLDN4*, *CLDN1*, and *OCLN* were substantially lower in TSCs and CSCs than in NSCs, and significantly lower in CSCs than in TSCs (A). However, NSCs and TSCs treated with 20 μM cortisol during differentiation showed no differences, except for an increase in TEER in TSCs at 72 h (B, C). A comparison of TSCs and CSCs treated with 20 μM cortisol during differentiation suggested that the increase in TEER was diminished for 24–72 h, and FITC-dextran permeation was significantly increased in CSCs (B, C).

Chronic and temporary stress reactions are affected differently by freshly supplied cortisol. Consequently, Chronic stress excessively attenuated barrier function more than temporary stress.

3. Influence of 11β HSD inhibitors for barrier function under cortisol



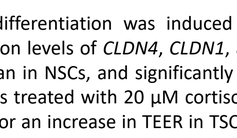
mean ± SEM, n = 3 , * $p < 0.05$, ** $p < 0.01$, vs. control using Dunnett's test.

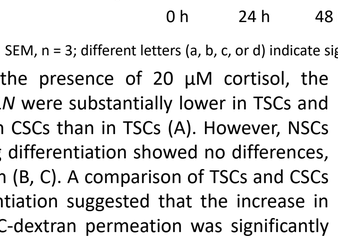
PF (11β HSD-1 inhibitor) did not increase the mRNA expression levels of TJPs in CSCs differentiated in the presence of cortisol (A). In contrast, DBP (11β HSD-2 inhibitor) decreased the mRNA expression levels of TJPs in TSCs differentiated in the presence of cortisol (B).

11β HSD-2 is more critical than 11β HSD-1 in the formation of the barrier function during differentiation in the presence of cortisol.

4. Screening of natural extracts that reduce the cortisol concentration under cortisone

Screening method:
Cortisone to cortisol conversion assay





GLSE decreased the cortisol concentration in the supernatant of NHEKs treated with 1 μM cortisone.

GLSE reduced the cortisol concentration in the supernatant of NHEKs.

mean ± SEM, n =3, ** $p < 0.01$, vs. control using Dunnett's test.

5. Promoting effect of GLSE or β-1,3-Glucan on formation of barrier function under cortisol

mean ± SEM, n =3, ** $p < 0.01$, vs. control using Dunnett's test.

GLSE significantly upregulated the mRNA expression level of CLDN4 at 72 h in CSCs differentiated in the presence of cortisol (A). GLSE and β-1,3-Glucan significantly increased the value of TEER (B, C).

GLSE and β-1,3-Glucan reduced the effects of cortisol in CSCs.

References:

1. Slominski A, Zbytek B, Nikolakis G, et al (2013) Steroidogenesis in the skin: implications for local immune functions. J Steroid Biochem Mol Biol 137:107-23.
2. May N B-J, Muhammad S N, Sadaf J G, et al (2022) Novelkaraya gum micro-particles loaded Ganoderma lucidum polysaccharide regulate sex hormones, oxidative stress and inflammatory cytokine levels in cadmium induced testicular toxicity in experimental animals. Int J Biol Macromol 194:338-346.
3. Liu Y, Wang Y, Zhou S, et al (2021) Structure and chain conformation of bioactive β-D-glucan purified from water extracts of Ganoderma lucidum unbroken spores. Int J Biol Macromol 180:484-493.

Acknowledgements:

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