To overcome insufficient sleep
– Screening of plant extracts that reinforce the effect of growth hormone in dermal fibroblasts –

SC_397

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Introduction:

GH-IGF axis

Insufficient sleep is an important issue for many people in modern society, and the risks of developing various diseases associated with sleep deprivation are considerable. **Growth hormone (GH)**, which is released from pituitary gland during deep sleep, primarily mediates growth-promoting action through the stimulation of **insulin-like growth factor-1 (IGF-1)** production in target tissues.

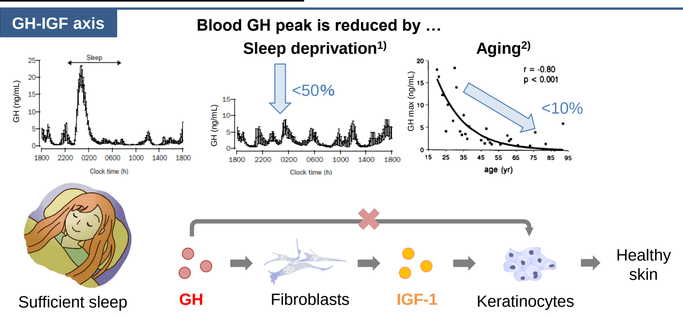
Insufficient sleep and skin

Sleep deprivation causes various physical symptoms by decreasing GH secretion and weakening the production of IGF-1 [1]. In addition, GH secretion has been reported to decrease with aging [2]. The characteristic physical signs of insufficient sleep are reflected in the facial skin. In the skin, dermal fibroblasts are GH target cells that produce IGF-1, which transmits information to keratinocytes to maintain healthy skin. GH deficiency is characterized by skin thinning and skin dryness. Although there are a few reports on the relationship between insufficient sleep and barrier function of the skin, effective measures to prevent the adverse effects of insufficient sleep on the skin have not been well studied.

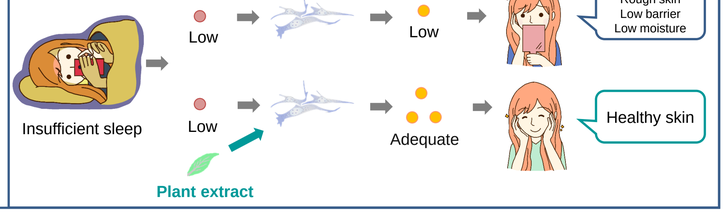
Objective

In the present study, we devised a new strategy for mitigating the effects of insufficient sleep by treating dermal fibroblasts with plant extracts. Specifically, we focused on IGF-1 secretion and searched for plant extracts that induce adequate IGF-1 expression at almost the same level as that with normal GH stimulation, even at a low amount of GH stimulation.

GH-IGF axis



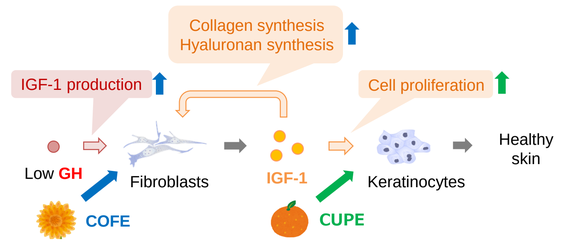
Hypothesis



Conclusions:

Summary

- Insufficient sleep model using NHDF-ad was prepared
- COFE reinforced the effect of GH in NHDF-ad
- IGF-1 promoted collagen and hyaluronan production in NHDF-ad
- COFE reinforced the effect of IGF-1 in NHDF-ad
- IGF-1 promoted cell proliferation in NHEKs
- CUPE reinforced the effect of IGF-1 in NHEKs



Daily application of COFE and CUPE can prevent skin problems by reinforcing GH effects on IGF-1 expression in fibroblasts, IGF-1 effects on collagen and hyaluronan production in fibroblasts, and IGF-1 effects on cell proliferation in keratinocytes, even under low GH secretion and conditions of insufficient sleep.

Conclusion

Even at a low GH level, COFE shows a comparable effect on dermal fibroblasts than that caused by high GH level in young skin or after sufficient sleep. The application of COFE advances a new strategy to alleviate the adverse effects on the skin when GH production declines due to aging or insufficient sleep.

Moreover, the application of CUPE, which reinforced the effect of IGF-1 on keratinocytes, is expected to alleviate the adverse effects on the skin during insufficient sleep and maintain healthy skin.

Materials & Methods:

Plant extracts

Calendula Officinalis Flower Extract (COFE)

Flowers of *Calendula officinalis*

Citrus Unshiu Peel Extract (CUPE)

Fruits of *Citrus unshiu*

Cell culture

Normal human dermal fibroblasts (NHDFs) derived adult donor (NHDF-ad, age: 36 years) were pre-cultured in Fibroblast Growth Medium 2 (FGM2). NHDF derived neonatal donor (NHDF-neo, age: 0-year, clone NB1RGB) and elderly donor (NHDF-old, age: 69 years, clone TIG-103) were pre-cultured DMEM with 10% fetal bovine serum (FBS). NHDFs were treated with sample in DMEM with 0.1% bovine serum albumin (BSA).

Normal human epidermal keratinocytes (NHEKs) were pre-cultured in keratinocyte growth medium (KGM) and were treated with sample in keratinocyte basal medium (KBM).

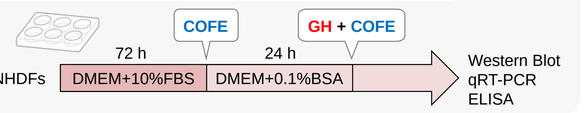
NHDFs

NHDF-neo (age 0 y)
NHDF-ad (age 36 y)
NHDF-old (age 69 y)

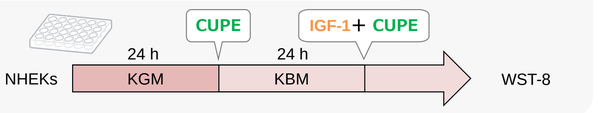
NHEKs

Test method

Treatment of NHDFs with GH and COFE or IGF-1



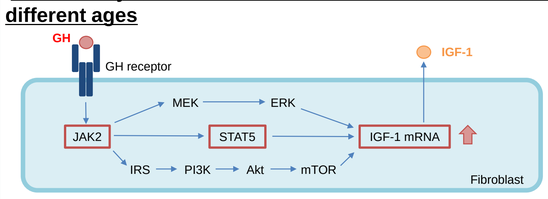
Treatment of NHEKs with IGF-1 and CUPE

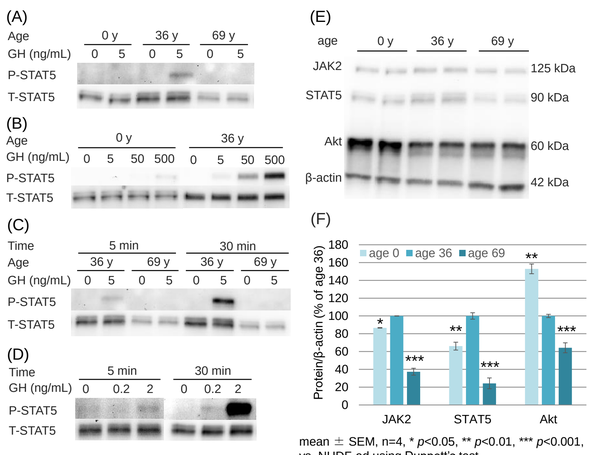


Results & Discussion:

Preparation of an insufficient sleep model *in vitro*

I. Reactivity of GH in NHDFs derived from individuals at different ages

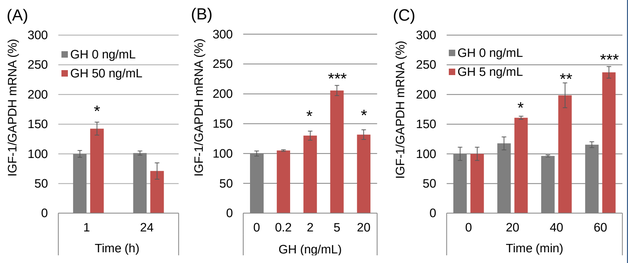




mean ± SEM, n=4, * p<0.05, ** p<0.01, *** p<0.001, vs. NHDF-ad using Dunnett's test.

STAT5 was only activated in NHDF-ad treated with 5 ng/mL GH at 5 minutes but not in NHDF-neo or NHDF-old (A). Activation of STAT5 in NHDF-ad treated with GH at 5 minutes was dose dependent and higher than that in NHDF-neo (B). Activation of STAT5 in NHDF-ad treated with 5 ng/mL GH increased until 30 minutes, but STAT5 was not activated in NHDF-old even after incubation for 30 minutes (C). The lowest activation dose of GH was 0.2 ng/mL at 30 minutes in NHDF-ad (D). The levels of JAK2 and STAT5 in NHDF-neo were lower than those in NHDF-ad, and the level of Akt in NHDF-neo was higher than that in NHDF-ad (E and F). The levels of JAK2, STAT5, and Akt in NHDF-old were lower than those in NHDF-ad (E and F).

II. Effect of GH treatment on IGF-1 mRNA expression in NHDF-ad



mean ± SEM, n=3, * p<0.05, ** p<0.01, *** p<0.001, vs. GH 0 ng/mL using t-test or Dunnett's test.

IGF-1 mRNA expression was promoted at 1 hour after treatment of NHDF-ad with GH but not at 24 hours after treatment (A). IGF-1 mRNA expression was increased in a dose-dependent manner up to 5 ng/mL at 1 hour (B) and in a time-dependent manner up to 60 minutes after the addition of GH (C).

- **STAT5 was only activated in NHDF-ad treated with GH.**
- **The lowest activation dose of GH was 0.2 ng/mL in NHDF-ad.**
- **T_{max} of IGF-1 mRNA expression was 1 hour after GH treatment.**
- **C_{max} of IGF-1 mRNA expression was 5 ng/mL GH.**

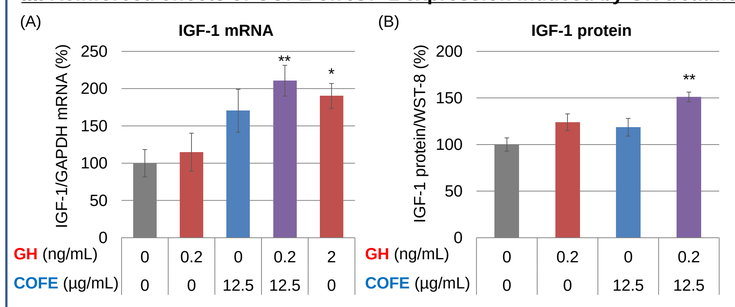
Blood GH peak is reduced less than half by sleep deprivation and less than one tenth by aging

Insufficient sleep model

IGF-1 mRNA expression is measured at 1 hour after treatment of NHDF-ad with 0.2 ng/mL GH.

Screening of plant extracts that reinforce the effect of GH in NHDF-ad

III. Reinforced effects of COFE on IGF-1 expression induced by GH treatment in NHDF-ad

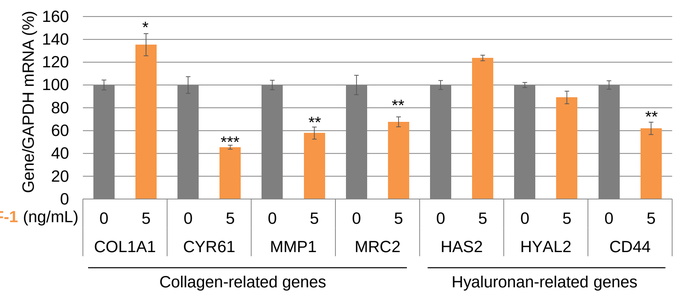


mean ± SEM, n=3, * p<0.05, ** p<0.01, vs. non-treatment using Dunnett's test.

The combined use of low levels of GH (0.2 ng/mL) and COFE produced a recovering effect on IGF-1 mRNA expression that was comparable to that after the appropriate GH (2 ng/mL) stimulation (A). The production of IGF-1 protein was also reinforced by the combination of low levels of GH and COFE (B).

In screening the effects of various plant extracts, we found that COFE reinforced IGF-1 mRNA expression and protein production during low-dose GH stimulation.

IV. Effects of IGF-1 on gene expression in NHDF-ad

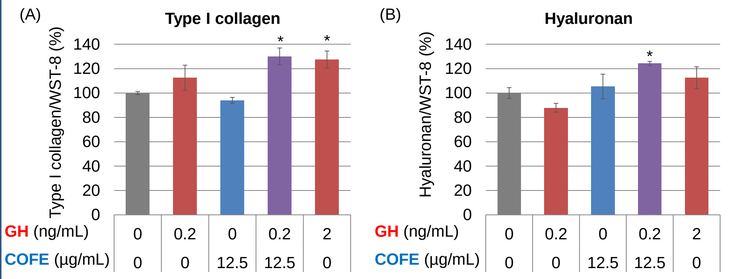


mean ± SEM, n=3, * p<0.05 , ** p<0.01 , *** p<0.001, vs. IGF-1 0 ng/mL using t-test.

In NHDF-ad, IGF-1 significantly upregulated mRNA expression of COL1A1 and tended to upregulate HAS2 (p=0.099), and downregulated CYR61, MMP1, MRC2, and CD44 at 24 hours .

To investigate the possibility that IGF-1 produced by fibroblasts acts on fibroblasts by autocrine action, IGF-1 was added to NHDF-ad. IGF-1 showed various effects on positively regulating collagen and hyaluronan content.

V. Reinforced effects of COFE on collagen and hyaluronan production induced by GH treatment in NHDF-ad



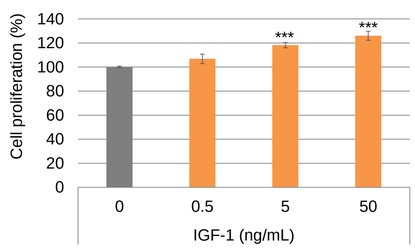
mean ± SEM, n=3, * p<0.05, vs. non-treatment using Dunnett's test.

The production of type I collagen (A) and hyaluronan (B) was reinforced by the combination of low levels of GH (0.2 ng/mL) and COFE.

COFE reinforced production of collagen and hyaluronan via promotion of IGF-1 production by GH.

Screening of plant extracts that reinforce the effect of IGF-1 in NHEKs

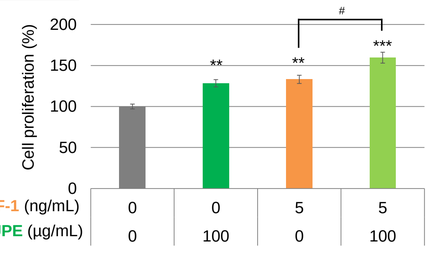
VI. Effect of IGF-1 on cell proliferation in NHEKs



mean ± SEM, n=6, *** p<0.001, vs. IGF-1 non-treatment using Dunnett's test.

IGF-1 promoted cell proliferation in a dose-dependent manner in NHEKs.

VII. Promoting effect of CUPE on cell proliferation induced by IGF-1 in NHEKs



mean ± SEM, n=3, ** p<0.01 , *** p<0.001, vs. non-treatment, # p<0.05 using Tukey's test.

The promotion of cell proliferation was reinforced by the combination of IGF-1 (5 ng/mL) and CUPE in NHEKs.

CUPE reinforced cell proliferation of NHEKs by IGF-1.

Acknowledgments:

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References:

1. Brandenberger G, Gronfier C, Chapotot F, Simon C, Piquard F (2000) Effect of sleep deprivation on overall 24 h growth-hormone secretion. *Lancet* 356:1408.
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