The molecular mechanism of PCE-DP, a novel brightening active ingredient

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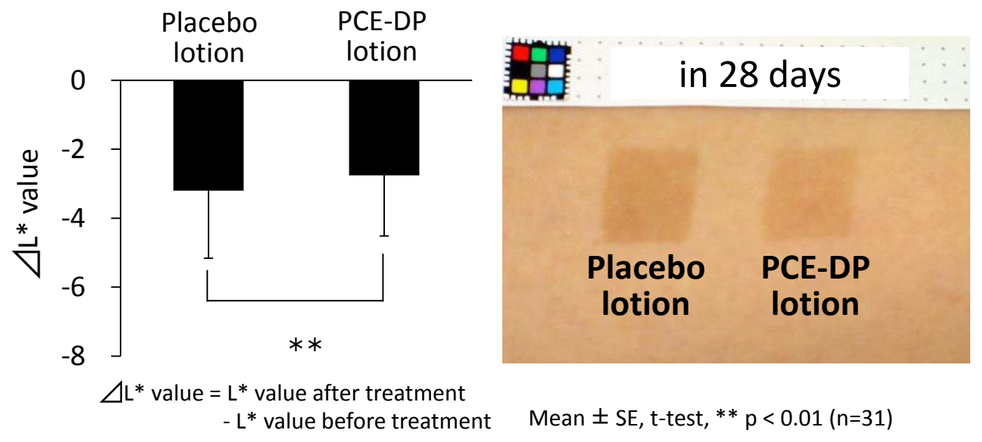
Introduction

PCE-DP (D-pantothenyl alcohol) is an active ingredient that prevents inflammation and promotes hair growth in pharmaceuticals and cosmetics.
In our previous randomized, double-blind placebo-controlled UVB irradiation-induced pigmentation study with healthy Japanese men and women, we found that topical treatment of PCE-DP-containing lotion resulted in brightening effects when used for 4 weeks.

Purpose: We performed four studies to clarify the molecular mechanism of PCE-DP activity regarding: (I) epidermal turnover, (II) melanosomal uptake by keratinocytes, (III) melanocyte activation by keratinocytes, and (IV) melanin production in melanocytes.

Inhibitory effects of PCE-DP on skin pigmentation by UVB irradiation



⊿L* value = L* value after treatment - L* value before treatment

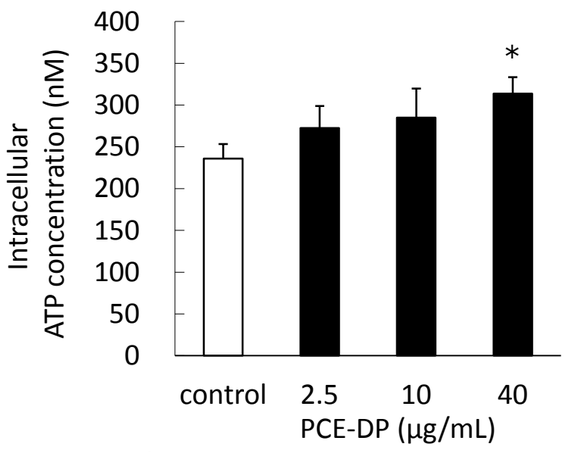
Mean ± SE, t-test, ** $p < 0.01$ (n=31)

Results

I. Epidermal turnover

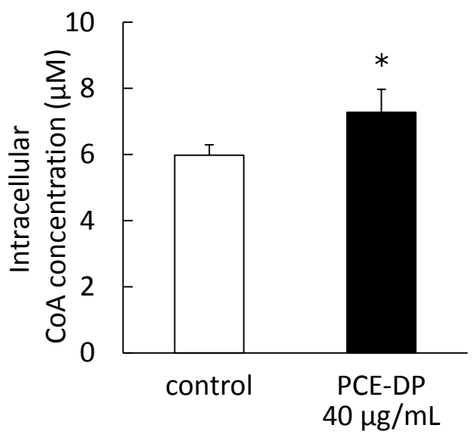
PCE-DP increases intracellular adenosine triphosphate (ATP) and coenzyme A (CoA) concentrations, and promotes cell growth in normal human keratinocytes (NHEK). It also promotes turnover in humans.

(A) ATP production



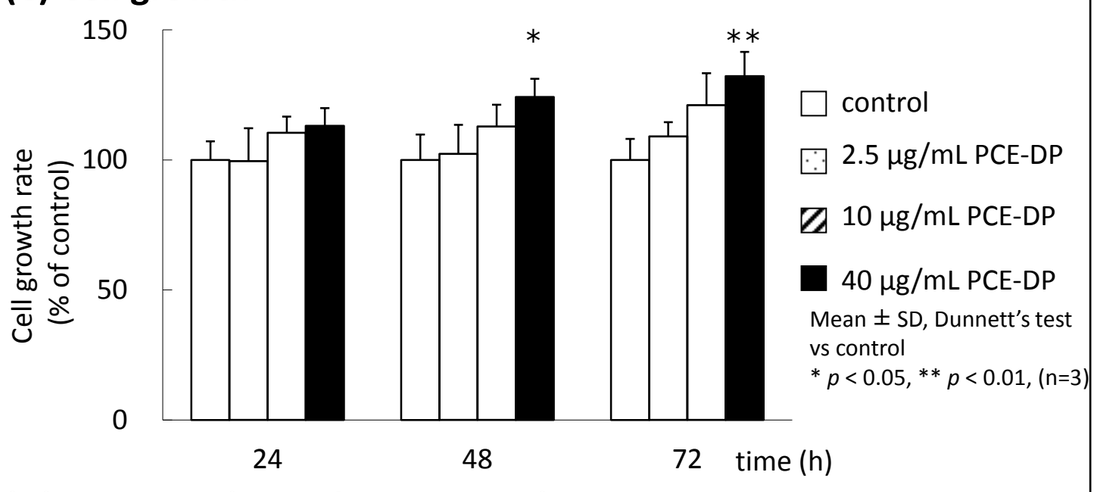
Mean ± SD, Dunnett's test vs control, * $p < 0.05$, (n=3)

(B) CoA concentration



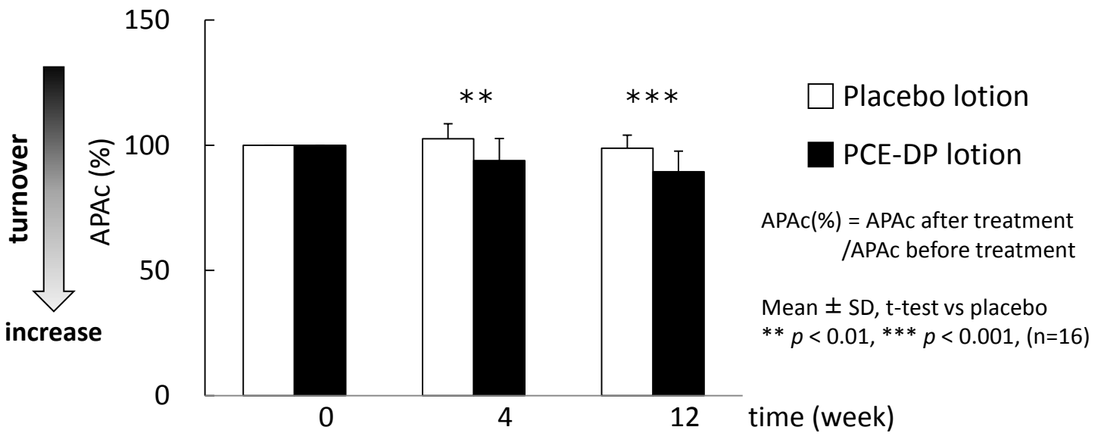
Mean ± SD, t- test , * $p < 0.05$, (n=3)

(C) Cell growth



Mean ± SD, Dunnett's test vs control
* $p < 0.05$, ** $p < 0.01$, (n=3)

(D) Turnover (size of corneocyte)



APAc(%) = APAc after treatment /APAc before treatment

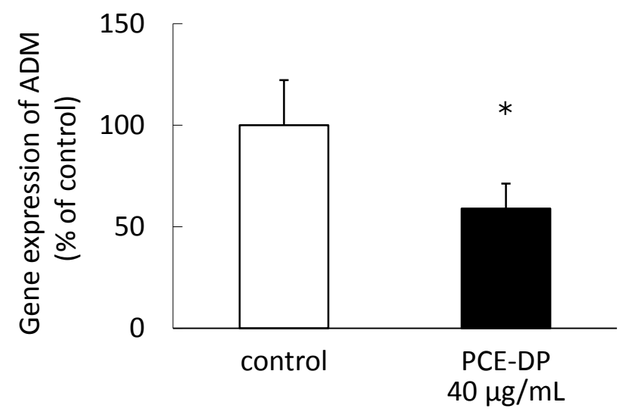
Mean ± SD, t-test vs placebo
** $p < 0.01$, *** $p < 0.001$, (n=16)

The effects of PCE-DP on (A) intracellular ATP, (B) intracellular CoA, and (C) cell growth of NHEK. (D) Effects on the average projected area of corneocytes (APAc) in healthy Japanese men and women. PCE-DP-containing lotion was applied on the medial upper arm three times a day for 12 weeks.

II. Melanosomal uptake by keratinocytes

PCE-DP reduced the mRNA expression of adrenomedullin (ADM), a keratinocyte-secreted key factor for dendrite elongation in melanocytes. In addition melanin uptake by keratinocytes was inhibited.

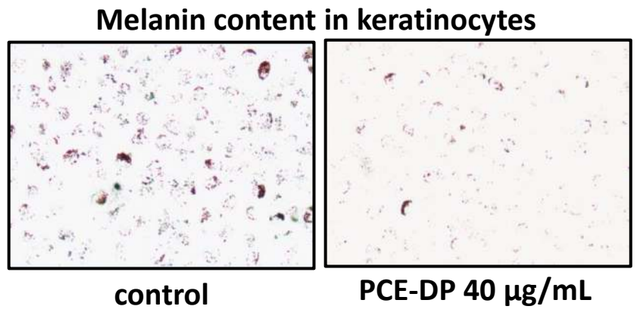
(A) ADM expression

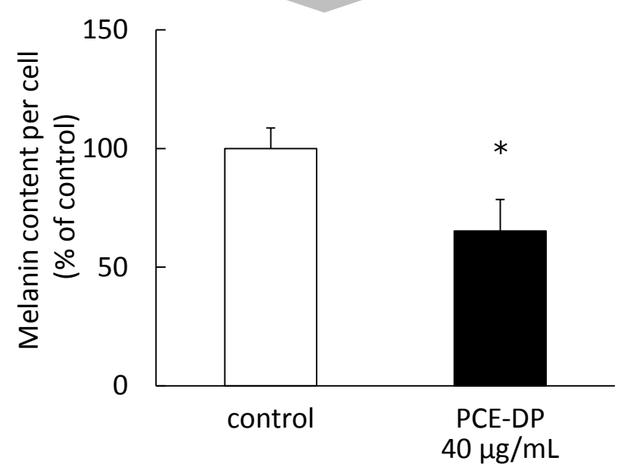


Mean ± SD, t- test , * $p < 0.05$, (n=3)

(B)Melanin uptake

Melanin content in keratinocytes





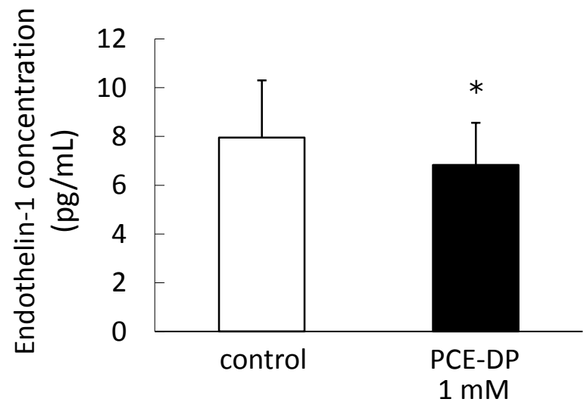
Mean ± SD, t- test , * $p < 0.05$, (n=3)

(A) ADM expression in UV-irradiated NHEK with PCE-DP.
(B) Melanin uptake in NHEK with PCE-DP.

III. Melanocyte activation by keratinocytes

PCE-DP reduced the keratinocyte-secreted endothelin-1 concentration, which increased melanin synthesis in melanocytes.

(A) Endothelin-1 concentration



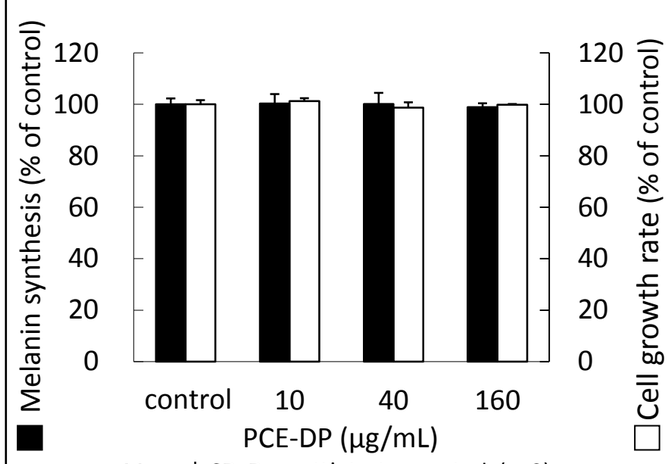
Mean ± SD, t- test , * $p < 0.05$, (n=6)

(A) Endothelin-1 concentration in UV-irradiated NHEK with PCE-DP.

IV. Melanin production in melanocytes

PCE-DP did not affect melanin synthesis or melanocyte growth.

(A) Melanin synthesis and melanocyte growth



Mean ± SD, Dunnett's test vs control, (n=3)

(A) Effects of PCE-DP on melanin synthesis and cell growth of Mc.

Methods

I. Evaluation of the effects of PCE-DP on turnover

A) Effects on increased intracellular ATP concentration in NHEK. The intracellular ATP concentration was measured using an ATP concentration measuring kit (ATP Bioluminescence Assay Kit HSII; Roche) after incubation of NHEK with PCE-DP solution for 6 hours.

B) Effects on increased intracellular CoA concentration in NHEK. The intracellular CoA concentration was measured using a CoA concentration measuring kit (Coenzyme A Colorimetric/Fluorometric Assay Kit; BioVision) after incubation of NHEK with PCE-DP solution for 6 hours.

C) Effects on cell growth in NHEK. After incubation of NHEK with PCE-DP solutions for 24, 48, or 72 hours, WST-8 reagents were added, and the absorbance was measured using a plate reader. The cell growth was then calculated.

D) Effects on the APAc. A double-blind, randomized, placebo-controlled study was conducted using healthy Japanese men and women. PCE-DP-containing lotion was applied on the medial upper arm three times a day for 12 weeks. The corneocytes were obtained by tape-stripping before treatment, and 4 and 12 weeks after treatment, and the APAc was calculated by image analysis software.

II. Evaluation of the effects of PCE-DP on melanosomal uptake by keratinocytes

A) Effects on the mRNA expression of ADM in NKEK. ADM mRNA in NHEK with PCE-DP solution was analyzed 6 hours after UV irradiation.

B) Effects on melanin uptake in NHEK. Melanosomes were extracted from B16 melanoma cells and added to NHEK. After a 24 hour incubation with PCE-DP solution, the cells were stained by Fontana-Masson. The size of intracellular melanosome aggregates was calculated using ImageJ software.

III. Evaluation of the inhibitory effects of PCE-DP on melanocyte activation by keratinocytes

A) Inhibitory effects of endothelin-1 production in NHEK. After 24 hours incubation with PCE-DP solution, The endothelin-1 concentration was measured using an endothelin-1 concentration measuring kit (Human Endothelin-1 Immunoassay; QuantiGlo) 9 hours after UV irradiation.

IV. Evaluation of the effects of PCE-DP on growth and melanin synthesis

A)-1 Effects on cell growth in melanocytes (Mc). After incubation of Mc with PCE-DP solutions for 3 days, WST-8 reagents were added and the absorbance was measured using a plate reader. The cell growth was then calculated.

A)-2 Effects on melanin synthesis by melanocytes (Mc). A ^{14}C-thiouracil incorporation assay was used. PCE-DP and ^{14}C-thiouracil were added to the medium. After 3 days, melanocytes were collected and the amount of intracellular ^{14}C-thiouracil incorporated was measured.

Conclusions

PCE-DP improved skin pigmentation by inducing epidermal turnover, which might be mediated by the activation of ATP production in NHEK. This improvement mechanism is thought to be via the tricarboxylic acid cycle through the increased intracellular CoA level. In addition, PCE-DP prevented skin pigmentation by inhibiting melanin uptake and inflammation after UVB irradiation in NHEK.