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LETTER TO THE EDITOR

WILEY Experimental Dermatology

PKCι is a target of 7,8,4′-trihydroxyisoflavone for the suppression of UVB-induced MMP-1 expression

ABSTRACT

The soy isoflavone daidzein is bioconverted to 7,8,4′-trihydroxyi soflavone (7,8,4′-THIF) by microorganisms. Here, we investigated the matrix metalloproteinase (MMP)-1 inhibitory properties of 7,8,4′-THIF that arise through the suppression of UVB-induced MMP-1 expression. 7,8,4′-THIF reduced UVB-induced MMP-1 expression at the transcriptional level in primary human dermal fibroblasts and inhibited UVB-induced transcriptional activity of AP-1, a major activator of MMP-1 expression. Additionally, it was observed that the mitogen-activated protein kinase (MAPK) pathway, a crucial signalling cascade for MMP-1 expression, was suppressed by 7,8,4′-THIF. Protein kinase C iota (PKCι) was suspected to be a direct target of 7,8,4′-THIF. The direct interaction between 7,8,4′-THIF and PKCι was confirmed using pull-down assays and immobilized metal ion affinity-based fluorescence polarization assays. Finally, we observed that 7,8,4′-THIF inhibited UVB-induced MMP-1 expression in a human skin equivalent model. Taken together, these results suggest that 7,8,4′-THIF, a bioconversion product of daidzein, suppresses UVB-induced MMP-1 expression.

1 | BACKGROUND

Daidzin (Fig. S1) is the most well-studied isoflavonoid in soybeans, and is present at approximately 118.5 mg per 100 g of dry weight.[1] Hepatic metabolism of daidzein yields the compound 7,8,4′-trihydroxyisoflavone (7,8,4′-THIF);[2] this compound is also found in aged Korean fermented soya paste, known as *doenjang*.[3]

Solar UV radiation is divided into three wavelength ranges: UVA (320-400 nm), UVB (280-320 nm), and UVC (200-280 nm). UVB is absorbed into the upper dermis of the skin and has numerous detrimental effects, including sunburn and immunosuppression. UVB exposure also has long-term consequences such as skin cancer and premature skin ageing, also known as photoaging.[4,5] Matrix metalloproteinase (MMP)-1 is the main MMP responsible for degradation of the extracellular matrix.[6,7] Repeated exposure to UVB can increase MMP-1 expression in dermal tissue through AP-1 activation,[8] and consequently promotes skin wrinkling.[6,9,10]

In a previous study, it was reported that the dominant negative mutant of PKCλ/ι suppressed UVB-induced AP-1 activation.[11] Additionally, other isotypes of PKC were reported to be regulators of various signalling pathways related to UVB-induced skin ageing.[12-14]

2 | QUESTIONS ADDRESSED

We investigated the MMP-1 inhibitory activity of 7,8,4′-THIF, the bioconversion product of daidzein. We report for the first time that 7,8,4′-THIF decreases UVB-induced MMP-1 expression by directly inhibiting PKCι kinase activity. These results suggest that 7,8,4′-THIF could be developed as an antiskin ageing cosmeceutical agent.

3 | EXPERIMENTAL DESIGN

3.1 | Human Skin equivalent preparation

Human skin equivalent (Neoderm-ED) was purchased from Tegoscience (Seoul, Korea) and incubated at 37°C in an atmosphere of 5% CO_2. After 2 weeks of air-lift, the human skin equivalent was treated with 7,8,4′-THIF (2.5, 5, or 10 μmol/L) or retinoic acid (10 μmol/L) for 1 hour as a positive control, and irradiated with UVB at 0.05 J/cm^2 once a day for 8 days. Medium was changed every 2 days during the incubation.

3.2 | Western blot analysis and zymography

Proteins were resolved by SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, MA, USA), which were then blocked with skim milk and hybridized with specific primary antibodies. The protein bands were visualized using an ECL solution after hybridization with a horseradish peroxidase-conjugated secondary antibody. The activity of MMP-2 was evaluated using zymography. Zymography was performed with 10% polyacrylamide gel in the presence of gelatin (0.5 mg/mL), which was used as a substrate for MMP-2.

3.3 | *In vitro* Kinase assay

PKCι kinase assay was performed using active recombinant PKCι enzymes following the manufacturer's instructions. Briefly, active PKCι and 7,8,4′-THIF were incubated at 30°C for 15 minutes in assay buffer. Two microlitres of myelin basic protein substrate was added to each mixture, and the mixtures were then incubated with [γ-32P] ATP solution in a magnesium acetate-ATP cocktail buffer (EMD Millipore)

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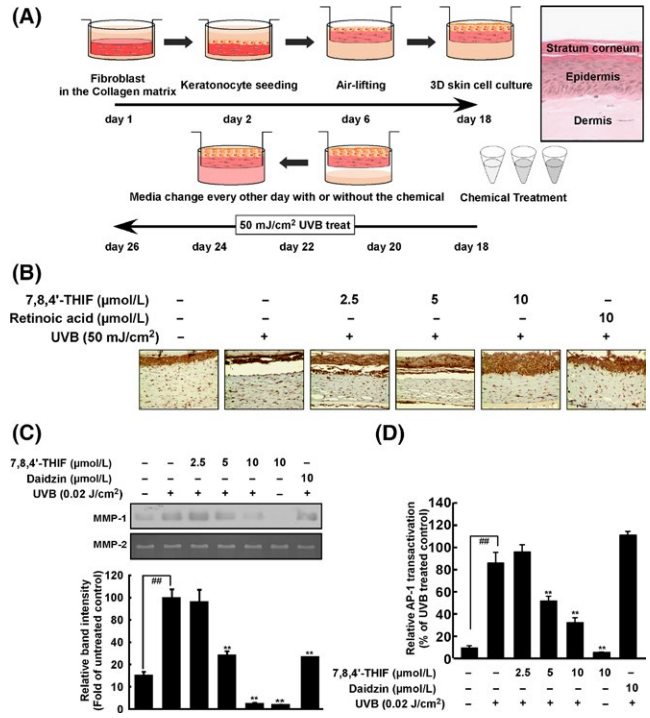

FIGURE 1 The inhibitory effect of 7,8,4′-THIF on MMP-1. (A) The procedure of 3D culture matrix preparation. The sample treatment and UVB irradiation were conducted as described in the Experimental design. (B) 7,8,4′-THIF reduces UVB-induced MMP-1 expression in 3D human skin equivalent model. The MMP-1 expression level was determined by immunohistochemistry using specific antibody. (C) 7,8,4′-THIF shows better inhibitory effect than daidzein on UVB-induced MMP-1 expression. After 48 hours of UVB irradiation, MMP-1 secretion by the cells was determined by western blot. MMP-2 was used as loading control. (D) 7,8,4′-THIF inhibits UVB-induced AP-1 transactivation. The asterisk (** and ##) indicates a significant difference (P<.001) compared to untreated control and UVB-treatment control, respectively. Data represented as the mean±SD

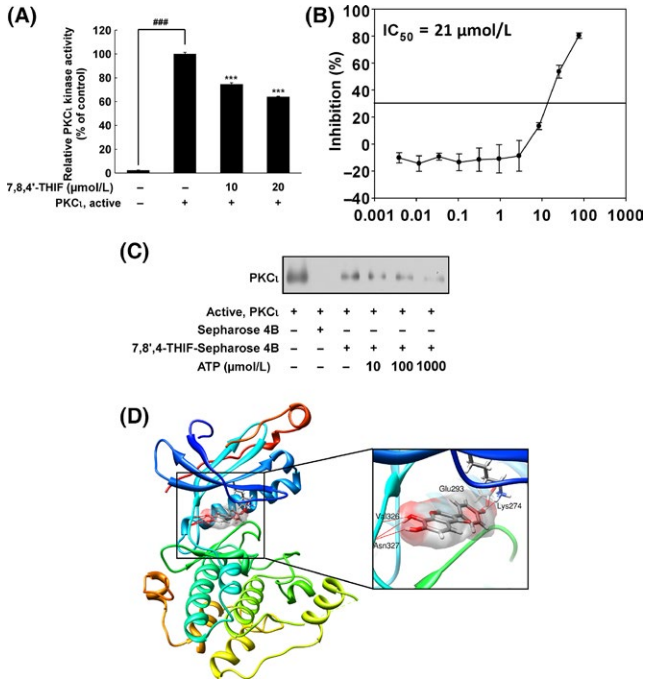

FIGURE 2 7,8,4′-THIF directly inhibits PKCι kinase activity. (A) PKCι was co-incubated with 7,8,4′-THIF at the indicated concentration for 1 hour at 30°C. After incubation with myelin basic protein, a substrate of PKCι, and [γ-^{32}P]ATP, the incorporated radiolabelled phosphate was measured using scintillation counter. The asterisk (*** and $^{###}$) indicates a significant difference (P<.05 and .001, respectively) compared to untreated control. (B) The Immobilized metal ion affinity-based fluorescence polarization (IMAP) assay was performed to estimate the IC_{50} value of 7,8,4′-THIF on PKCι kinase activity. (C) 7,8,4′-THIF binds to ATP pocket of PKCι. (D) The hypothetical binding pose of 7,8,4′-THIF (Val^{326} and Asn^{327} at backbone; Lys^{274} and Glu^{293} at side chain) the ATP-binding site of PKCι

at 30°C for 15 minutes. The radioactively labelled phosphate was measured using a scintillation counter.

3.4 | Statistical analysis

Data are expressed as the mean±standard deviation (SD). Student's t-test was used to evaluate the mean differences between groups and statistical significance. Differences were considered significant at P<.05.

4 | RESULTS

We tested the activity of 7,8,4′-THIF in a 3D human skin equivalent model, which has been reported to be more physiologically accurate than monolayer cultures. We investigated whether 7,8,4′-THIF could reduce UVB-induced MMP-1 expression in the human skin equivalent model (Figure 1A). UVB exposure increased MMP-1 expression in the epidermis of the human skin equivalent model (Figure 1B), and 7,8,4′-THIF inhibited UVB-induced MMP-1 expression. Surprisingly, 7,8,4′-THIF exhibited greater MMP-1 inhibitory activity than that of the well-known MMP-1 inhibitor retinoic acid. Additionally, we sought to compare the effect of 7,8,4′-THIF and its natural precursor daidzein on UVB-induced MMP-1 expression in primary human dermal fibroblasts. Treatment with 7,8,4′-THIF was observed to dose-dependently decrease UVB-induced MMP-1 expression (Figure 1C). The activity of the major transcription factor AP-1 was attenuated by 7,8,4′-THIF, as seen in Figure 1D. Additionally, the activity of the MAPK signalling pathway (the upstream signalling pathway of AP-1) was decreased by treatment with 7,8,4′-THIF (Fig. S2).

The kinase array in Fig. S3 shows that PKCι may be a target protein of 7,8,4′-THIF". As shown in Figure 2A, a dose-dependent reduction in PKCι kinase activity was observed following treatment with 7,8,4′-THIF. We calculated IC_{50} values for 7,8,4′-THIF using an immobilized metal ion affinity-based fluorescence polarization (IMAP) assay system.[15] The IC_{50} value of 7,8,4′-THIF for PKCι kinase activity was determined to be 21 μmol/L (Figure 2B). To determine whether 7,8,4′-THIF binds to the ATP-binding site of PKCι, we added varying concentrations of ATP (10, 100, and 1000 μmol/L) to PKCι in the assay before

7,8,4′-THIF treatment. As seen in Figure 2B, the binding between PKCι and 7,8,4′-THIF was diminished by ATP in a dose-dependent manner. PKCι was shown to be an upstream kinase of MAPK using a knockdown PKCι system (Fig. S4). We created a computational docking model using the Glide docking software in Schrödinger Suite 2012. Key hydrogen bonds formed between 7,8,4′-THIF and PKCι (Val^{326} and Asn^{327} at the backbone; Lys^{274} and Glu^{293} at the side chain) (Figure 2D). This supports the notion that 7,8,4′-THIF acts as an inhibitor of PKCι.[16]

5 | CONCLUSIONS

In this study, we investigated the MMP-1 inhibitory activity of 7,8,4′-THIF, the bioconversion product of daidzein. We report for the first time that 7,8,4′-THIF decreases UVB-induced MMP-1 expression by directly inhibiting PKCι kinase activity. These results suggest that 7,8,4′-THIF could be developed as an antiskin ageing cosmeceutical agent.

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CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTION

T.G. Lim and Y.A Kim performed in vitro experiments and analysed the data. J.E. Kim and C. Lee wrote the manuscript. S. Baek performed IMAP assay. J.R. Kim, S.Y. Lee, A.M. Bode and J.Y. Kwon reviewed the manuscript. H. Chen provided the computer modelling result. J.S. Park provided 7,8,4′-THIF. Z. Dong, C.Y. Lee and K.W. Lee supervised the experimental procedure.

Keywords
3D culture, 7,8,4′-trihydroxyisoflavone, matrix metalloproteinase-1, Protein kinase C iota

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. Chemicals structure and the cell viability

Figure S2. 7,8,4′-THIF suppressed phosphorylation of MEK-ERK, MKK4-JNK and MKK3/6-p38 phosphorylation

Figure S3. Kinase profiling analysis of 7,8,4′-THIF by KinaseProfilerTM service (MERCK Millipore). And the direct interaction between 7,8,4′-THIF and PKCι

Figure S4. PKCι regulates solar UV-induced MMP-1 expression as a upstream kinase of MAPKK signalling pathway