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Flt3 is a target of coumestrol in protecting against UVB-induced skin photoaging

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ABSTRACT

While skin aging is a naturally occurring process by senescence, exposure to ultraviolet (UV) radiation accelerates wrinkle formation and sagging of skin. UV induces skin aging by degrading collagen via activating matrix metalloproteinases (MMPs). In this study, we show that coumestrol, a metabolite of the soybean isoflavone daidzein, has a preventive effect on skin photoaging in three-dimensional human skin equivalent model. Coumestrol inhibited UVB-induced MMP-1 expression and activity. Whole human kinase profiling assay identified FLT3 kinase as a novel target protein of coumestrol in UVB-induced signaling pathway in skin. Coumestrol suppresses FLT3 kinase activity, and subsequently, Ras/MEK/ERK and Akt/p70 ribosomal S6 kinase pathway. This suppresses AP-1 activity and in turn, diminishes MMP-1 gene transcription. Using X-ray crystallography, the binding of coumestrol to FLT3 was defined and implied ATP-competitive inhibition. Residues Lys644 and Phe830 showed local changes to accommodate coumestrol in the ATP-binding pocket. 4-APIA, a pharmacological inhibitor of FLT3, inhibited MMP-1 expression and induced signal transduction changes similar to coumestrol. Taken together, coumestrol inhibits UVB-induced MMP-1 expression by suppressing FLT3 kinase activity. These findings suggest that coumestrol is a novel dietary compound with potential application in preventing and improving UVB-associated skin aging.

1. Introduction

Skin aging is a naturally occurring process by senescence, but is also affected by environmental stress such as sunlight, air pollution, and extreme temperature which causes premature skin aging [1,2]. In particular, ultraviolet (UV)B radiation from the sunlight is the major cause of exacerbated skin aging and leads to skin damage such as sunburn, immune-suppression, photoaging [3], and photo-carcinogenesis [4].

Exposure to UVB alters biological processes that promote matrix metalloproteinases (MMPs) expression, decrease procollagen synthesis, and increase connective tissue damage [2,5,6]. These complex changes trigger wrinkle formation throughout the various layers of skin, but the major changes occur in the dermis [7,8]. MMP-1 is a type of collagenase, which breaks down collagen fibrils. Repeated UVB exposure increases the level of MMP-1 in the dermis and dermal fibroblasts, and therefore triggers histopathological changes [5,6,8]. MMP-1 expression is mediated by cellular

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signaling transduction such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt [9,10] pathways. These signaling cascades elevate activator protein 1 (AP-1) activity and enhance MMP-1 gene transcription.

Coumestrol (Fig. 1A), one of the soybean isoflavonoids, belongs to the coumestan family [11,12]. Under stress such as germination, fungal infection, or chemical elicitors, coumestrol is produced as a phytoalexin through the conversion of daidzein (Fig. 1B), the aglycon of daidzin (Fig. 1C) [13,14]. Although less studied, coumestrol has a higher antioxidant activity than the major soybean isoflavones including daidzein and genistein [15–17]. Recent reports showed that coumestrol exerts anti-cancer, anti-obesity, and neuroprotective effects without significant side effects [18–20]. In this study, we investigated the potential protective effect of coumestrol against UVB-induced skin photoaging and aimed to uncover the direct molecular target of coumestrol.

2. Materials and methods

2.1. Chemicals and reagents

Coumestrol, daidzin, daidzein, FBS, and β-actin antibody were obtained from Sigma–Aldrich (St. Louis, MO). DMEM was purchased from Hycolne (Long, UT). MMP-1 antibody was obtained from R&D Systems Inc. (Minneapolis, MN). Antibodies against phosphorylated ERK1/2 (Thr^{202}/Ty^{r204}) and total ERK1/2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The other antibodies were obtained from Cell Signaling Biotechnology (Danvers, MA). Penicillin/streptomycin was purchased from Life technologies (Carlsbad, CA). 4-(4-Aminophenyl)-1H-indazol-3-ylamine (3-aminoindazole compound) was obtained from MERCK Millipore (Nottingham, UK).

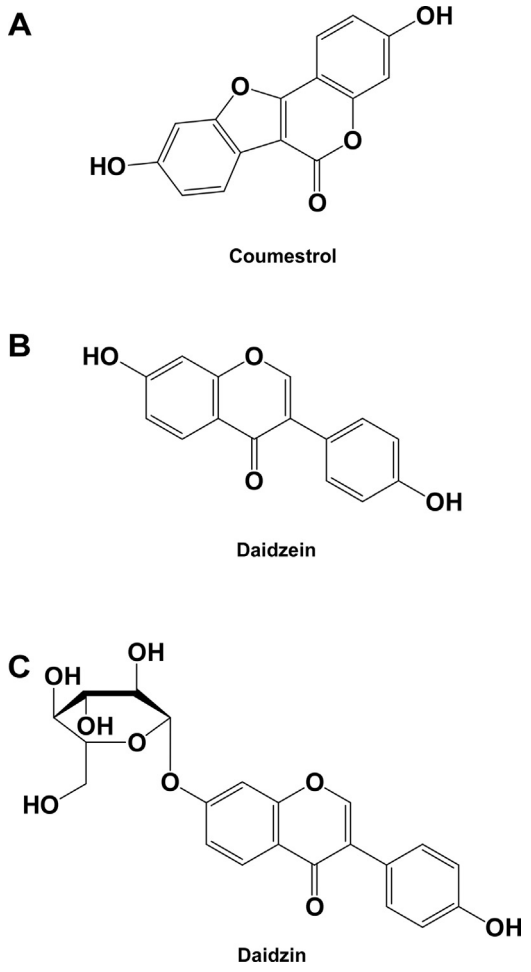

Fig. 1. Chemical structure of coumestrol, daidzin, and daidzein.

2.2. Cell culture and UVB irradiation

Human dermal fibroblasts (HDFs) were isolated from the outgrowth of foreskin obtained from 7 to 30 year old healthy volunteers from Dr. J.H. Chung's laboratory at Seoul National University (SNU) Hospital (Seoul, South Korea) under the Institutional Review Board at SNU Hospital and SNU. HDFs were cultured in DMEM with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin at 37 °C and 5% CO_2. Serum-starved monolayer cultures of HDFs were exposed to UVB at a dose of 0.02 J/cm^2 using UVB source (Bio-Link crosslinker, Vilber Lourmat, Cedex 1, France) set spectral peak at 312 nm.

2.3. 3D human skin equivalent system

Using Neoderm®-ED purchased from TEGO Science (Seoul, South Korea), we generated the 3D human skin equivalent system. Briefly, HDFs were cultured in collagen matrix for 1 day. Keratinocytes were then seeded on top of collagen matrix and co-cultured for 4 days. Next, the keratinocytes and HDF blocks were lifted and allowed to be exposed to air. Coumestrol, daidzin, and daidzein were treated for 1 h after 2 weeks of the air-lift. Prepared 3D human skin blocks were irradiated with 0.05 J/cm^2 UVB twice a day for 8 days. During this period, the medium was changed every 2 days and the blocks were incubated at 37 °C and 5% in a CO_2 atmosphere.

2.4. Cell viability

The cell viability was measured using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) following the manufacturer's instructions.

2.5. Histological examination

3D human skin equivalent blocks were fixed with 10% neutral-buffered formalin, and embedded in paraffin. We sectioned the paraffin block (4 μm thickness) and transferred them onto slides. After deparaffinization, the sections were stained with hematoxylin and washed and stained in biebrich scarlet and acid fuchsin. Next, the slides were placed in phosphomolybdic–phosphotungstic acid and then in aniline blue to stain collagen, and washed and incubated in 1% acetic acid. After dehydration and washing, the sections were examined at 400× magnification using an Olympus AX70 light microscope (Tokyo, Japan).

2.6. Immunohistochemical staining

3D human skin equivalent blocks were fixed, embedded, and deparaffinized as described above. The slides were incubated in 0.3% hydrogen peroxide to remove the endogenous peroxidases and blocked using 5% normal goat serum. After blocking, the slides were incubated with MMP-1 antibody at 4 °C overnight. Next, they were reacted to biotinylated secondary antibody (Vector Labs, Burlingame, CA) and developed using avidin–biotin complex kit (Vector Labs). The reaction was visualized with 3,3′-diaminobenzidine tetrahydrochloride hydrate solution (Vector Labs). The counterstain was conducted using hematoxylin. The level of MMP-1 was examined under 400× magnification. To evaluate collagen status in the dermis, Masson's trichrome staining was performed. Mouse skin samples and human skin equivalents were fixed with