Journal of Photochemistry & Photobiology, B: Biology 178 (2018) 92–100

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Consumption of ellagic acid and dihydromyricetin synergistically protects against UV-B induced photoaging, possibly by activating both TGF-β1 and wnt signaling pathways

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ARTICLE INFO

Keywords:
Photo-damage
Oxidative stress
Inflammation
TGF-β1 signaling
Wnt signaling

ABSTRACT

Ellagic acid (EGA) and dihydromyricetin (DHM) are both found in fruits and vegetables are used for anti-aging treatment for the skin. The anti-photoaging efficacy of EGA and DHM was investigated in UV-B irradiated skin in vivo and the involvement of transforming growth factor (TGF)-β1 and wnt signaling pathways were examined in vitro. HaCaT cells were treated with either 50 μM EGA, 50 μM DHM or 25 μM EGA + 25 μM DHM before 100 mJ/cm^2 UV-B exposure, and then oxidative stress and inflammation was measured. The involvement of TGF-β1 and wnt signaling was measured using their inhibitors, respectively, in HaCaT cells. Mice were fed a high fat diet with either 0.7% cellulose, 0.7% EGA, 0.7% DHM or 0.35% EGA + 0.35% DHM for 3 weeks and the dorsal skin of the mice had UV-B irradiation. 3% cellulose, 3% EGA, 3% DHM or 1.5% EGA + 1.5% DHM in 1,3-buthylene glycol was applied onto the dorsal skin at 30 min before 1 MED UV-B exposure. In 100 mJ/cm^2 UVB irradiation, EGA and DHM mainly decreased oxidative stress and inflammation, respectively in HaCaT cells. Their activities were blocked by the TGF-β1 inhibitor, indicating their actions were mediated by TGF-β1 signaling (TGF-β1 ➔ pSmad3 ➔ Smad7). DHM enhanced wnt signaling by increasing β-catenin and decreasing Dickkopf-related protein-1. In mice, 1 MED UV-B exposure induced sunburn, redness, and blistering. EGA, DHM and especially EGA + DHM lessened their severity. UV-B increased epidermal thickness and damaged epidermal nucleus and cell structures. DHM and especially EGA + DHM prevented damage to the nucleus and cell structures. Expressions of circulating and dorsal skin IL-1β and TNF-α mRNA were lower in descending order of: control, EGA, DHM, EGA + DHM and normal-control. In conclusion, the consumption of EGA + DHM had a synergistically protective action against UV-B damage in the skin tissues of mice and HaCaT cells, and it may be associated with activating of both TGF-β1 and wnt signaling.

1. Introduction

People are increasingly concerned about skin aging as society ages, Aging is accelerated by various factors such as ultraviolet (UV) light, smoking, and pollutants. Among these factors, UV light exposure is one of the major factors that accelerate aging in the skin [1]. The skin has an important function to protect the body from foreign attacks such as from pathogens and pollutants [1,2]. The skin barriers need to be maintained in an intact form. Sun exposure has beneficial effects on body function such as synthesis of vitamin D [3,4], but chronic sun over exposure causes sunburn, tanning, winkles, photo-aging and photo-damage by UV light including UV-A (315–400 nm) and UV-B (280–315 nm) [1]. UVB is completely absorbed by the epidermis with high energy and directly damages the DNA of the epidermis including keratinocytes, whereas UVA radiation penetrates deeper into the skin and damages dermal fibroblasts indirectly [2]. Chronic UV radiation changes the collagen-rich extracellular matrix by increasing various metalloproteinases (MMP) which break down the extracellular matrix. It reduces dermal collagen contents and increases the expression and activity of MMP-1 that makes the degradation of interstitial collagen [5]. In addition, photoaging activates oxidative stress and inflammation [1]. UV stimulates the signaling transcription of nuclear factor kappa B (NF-κB), which activates the synthesis of cytokines that promotes the synthesis of reactive oxygen species (ROS) [5]. Chronic UV exposure creates a vicious cycle which exacerbate oxidative stress and inflammation thereby causing deterioration of the dermal tissues. The

Abbreviations: MMP-1, matrix metalloproteinase-1; UV, ultraviolet; EGA, ellagic acid; DHM, dihydromericetin; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; SOD, superoxide dismutase; GSH, glutathione; Px, peroxidase; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; MED, minimal erythema dose; PCR, polymerase chain reaction; DKK1, dickkopf-related protein-1; NO, nitric oxide; iNOS, inducible nitric oxide synthase

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https://doi.org/10.1016/j.jphotobiol.2017.11.004
Received 30 August 2017; Received in revised form 30 October 2017; Accepted 2 November 2017
Available online 07 November 2017
1011-1344/

most common solution to reduce the deleterious actions of UV exposure is to apply sunscreen. However, sunscreen has potential risks since the major ingredients such titanium dioxide, zinc oxide and p-aminobenzoic acid have adverse effects [6]. Photoaging should be suppressed using natural products that reduce oxidative stress and inflammation instead of using sunscreen [6].

Polyphenols in vegetables and fruits have been reported to have anti-photoaging activity [7,8]. Ellagic acid (EGA), a dilactone of hexahydroxydiphenic acid, and dihydromyricetin (DHM), a flavanonol, most effectively protected against cell death from UV-B exposure among several polyphenols screened in our preliminary study. EGA, which occurs in high concentration in pomegranate, protects against oxidative stress, viruses, bacteria, inflammation, and carcinogens [9]. Its anti-oxidant and anti-inflammatory activities remove the increased ROS and cytokines, thereby protecting against photoaging due to UV exposure. EGA stimulates anti-oxidative enzyme system including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase and catalase [10]. In human dermal fibroblast cells, ellagic acid decreased ROS and MMP-2 expressions induced by UV exposure, while restoring total GSH and SOD, important anti-oxidative enzymes [11]. Myricetin, another flavonoid, suppresses the phosphorylation of extracellular signal–regulated kinases and mitogen-activated protein kinase and MMP-9 expression induced by UV exposure in mice [12]. In addition, DHM, which has a similar chemical structure to myricetin, has also exhibited anti-inflammatory and anti-oxidant activities [13,14]. Recent studies have demonstrated that DHM has higher bioavailability than myricetin in gut intestinal tract [15]. Although it has not been studied in anti-photoaging, DHM may have a better anti-photoaging activity than myricetin.

The mechanism(s) involved the anti-photoaging actions remain obscure, UV exposure is known to decrease collagen synthesis and wound healing. The proliferation and differentiation of skin tissues are associated with transforming growth factor-β (TGF-β) and wnt signaling pathways [16]. UV exposure inhibits type I procollagen synthesis by down-regulation of type II receptor of TGF-β1 and up-regulating Smad7 [16]. Therefore, we determined the efficacy of EGA and DHM for anti-photoaging properties under UV-B irradiation using in vitro and in vivo studies, and the mechanism involved in TGF-β1 and wnt signaling pathways was explored in vitro.

2. Materials and Method

2.1. Cell Culture

HaCaT cells, a human keratinocyte cell line, were cultured with DMEM supplemented with 10% FBS, 100 units/ml of penicillin, and 100 μg/ml of streptomycin in a humidified atmosphere of 5% CO_2 in 95% air at 37 °C. HaCaT cells were exposed to 100 mJ/cm^2 UV-B TL20W/12 RS SLV/25 lamp (Philips, Amsterdam, Netherlands) with an emission spectrum between 275 and 380 nm (310–315 nm at peak). The UV light intensity was checked using a UV illuminance meter (EC1-X UV-B Digital Radiometer, Hagner, Solna, Sweden) with a peak emission at 312 nm. In our preliminary study, cell death was accelerated in HaCaT cells exposed to 50 mJ/cm^2 MED or more, in a dose-dependent manner. Since 100 mJ/cm^2 UV-B exposure sufficiently induced photoaging, 100 mJ/cm^2 UV-B was used for exploring the protective effects of herbs and polyphenols against photoaging.

The HaCaT cells were treated with DMSO (control), 50 μM EGA, 50 μM DHM or 25 μM EGA + 25 μM DHM at 30 min before UV-B exposure. Normal-controls were treated with DMSO under a fluorescent light. The optimal dosages for EGA and DHM was determined in our preliminary study. Cells were exposed to either 0, 50, 100 or 200 mJ/cm^2 UV-B, and cells were further incubated in fresh medium in the presence of the assigned EGA and DHM. After 12 h treatment, viability of the cells was determined in triplicate by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The live cells metabolized MTT by mitochondrial dehydrogenases to make a purple formazan dye and the color change was measured at 550 nm. Lipid peroxide levels in the cell were detected by using a thiobarbituric acid reactive substances kit. ROS in the cells were measured by reacting with 2.7-dichlorofluorescin diacetate (Sigma Co., St. Louise, MO, USA). Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels in the media were measured with ELISA kits (R & D Systems, Minneapolis, MN, USA and Amersham Biosciences, Piscataway, NJ, respectively). The cells were harvested and lysed with lysis buffer. Lipid peroxides levels were measured in the lysates. In addition, the cDNA was synthesized from the treated cells and mRNA expression of TNF-α, IL-1β, SOD, MMP-1, TGF-β1 and collagen I were measured with realtime PCR as described below. In order to explore the mechanism of EGA and DHM, HaCaT cells were pretreated with 10 μM SB431542 (TGF-β1 signaling inhibitor) or XAV939 (wnt signaling inhibitor) at 30 min before EGA and DHM and then 30 min later the cells were irradiated with UV-B. After 12 h, mRNA expressions of TGF-β1, Smad7, β-catenin, and dickkopf-related protein-1 (DKK1) were measured using realtime PCR.

2.2. Animals

Fifty male 6-week-old ICR mice were purchased from Dae Han Biolink (Eum Sung, Korea) and they were acclimated in an environmentally controlled animal facility with a 12 h light/12 h dark cycle, room temperature of 22–23 °C and humidity of 55 ± 15%. The mice had free access to food and water. All surgical and experimental procedures were performed according to the guidelines and with the approval of the Animal Care and Use Review Committee at Hoseo University, Korea (2013-05).

2.3. UV-B Exposure and Treatment of Mice

High fat diets were used in the present study since the diets exacerbated oxidative stress which was generated with UV-B exposure compared with a low fat diet. The high fat diet was a semi-purified modified AIN-93 formulation containing 37 energy percent (En%) from carbohydrates, 20 En% from protein and 43 En% from fats. The sources of carbohydrate, protein and fat were starch plus sugar, casein (milk protein) and lard (CJ Co., Seoul, Korea), respectively. The control and normal-control diets included 0.7% cellulose in a high fat diet and 0.7% ellagic acid, 0.7% dihydromyricetin and 0.35% ellagic acid + 0.35% dihydromyricetin was supplemented instead of cellulose for EGA, DHM and EGA + DHM groups, respectively. The lotions containing cellulose, EGA, DHM or EGA + DHM were prepared by dissolving them into 1,3-buthylene glycol to make a 3% solution for application into the dorsal skin for the assigned group. The 1,3-butylene glycol containing cellulose was made for the control and normal-control groups.

The mice were divided into the control, EGA, DHM, and EGA + DHM groups of 10 mice each and they had the assigned diet for 3 weeks prior to UV-B exposure. Ten mice in the normal-control group had a high fat diet for 3 weeks without UV-B exposure. The hair in the back was removed after anesthesia with a mixture of ketamine and zylazine (100 and 10 mg/kg body weight). The next day the mice had the same assigned same flavonoid applied to the dorsal skin and then 30 min later the dorsal skin of the mice were exposed to 1 minimal erythema dose (MED) of UV-B. MED is a useful biological read-out system to induce a slight redness of the skin. 1 MED was calculated as 100 mJ/cm^2 when the UV light was placed at 30 cm above the plate. The mice were further provided with the assigned diet for 1 more week to explore the efficacy of the flavonoids in healing the damage. The assigned lotion was applied once before UV-B exposure and the application was discontinued.

2.4. Evaluation of Skin Lesion

The relative severity of photoaging in the dorsal skin was clinically