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Rubus idaeus L. (red raspberry) blocks UVB-induced MMP production and promotes type I procollagen synthesis via inhibition of MAPK/AP-1, NF-κβ and stimulation of TGF-β/Smad, Nrf2 in normal human dermal fibroblasts

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ABSTRACT

Chronic exposure to ultraviolet (UV) radiation causes photo-oxidation, which in turn results in the upregulation of matrix metalloproteinases (MMPs) and loss of collagen. *Rubus idaeus* L. (RI), also called red raspberry, is an important cash crop that contains abundant antioxidant compounds. Sanguiin H-6 and lambertianin C are the major ingredients presented in the extracts. Here, we studied the protective effect of RI on UVB-induced photoaging in normal human dermal fibroblasts (NHDFs). We found that RI notably reduced UVB-induced MMPs secretion and pro-inflammatory mediators production, and significantly suppressed UVB-induced activation of mitogen-activated protein kinase (MAPK), nuclear factor-κβ, as well as activator protein 1. Additionally, treatment of NHDFs with the ERK inhibitor (PD98059) and JNK inhibitor (SP600125) resulted in the reduction of UVB-induced MMP-1 and IL-6 expressions, which demonstrated that the inhibition of MMP-1 and IL-6 by RI is associated with the MAPK pathway. Furthermore, we also found that RI accelerated procollagen type I synthesis by activating the transforming growth factor-β/Smad pathway and enhanced the expression of cytoprotective antioxidants such as heme oxygenase-1 and NHD(P)H quinone oxidoreductase 1 by promoting nuclear factor E2-related factor 2 nuclear transfer. Overall, these findings demonstrated that RI was potentially effective in preventing UVB induced skin photoaging.

1. Introduction

Skin is the major physiological barrier that protects our body from external injuries. Ultraviolet (UV) irradiation from the sun is generally considered to be one of the most hazardous environmental factors for our skin. UV radiation can be divided into three categories: UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm). UVC is completely blocked by the ozone layer, while UVA and UVB can reach the surface of the earth and do harm to human health [1]. The electromagnetic energy from both UVA and UVB can be absorbed by cellular chromophores. These energized chromophores will react with molecular oxygen, leading to the generation of reactive oxygen species (ROS). Excessive levels ROS have the capacity to alter elastic and collagen fibers of connective tissue in dermis, and finally resulting in photoaging [2]. UV-induced photoaging is characterized by a leathery appearance, wrinkles formation and less elasticity [3].

Photoaging is a long-term cumulative process that mainly happens in skin dermal connective tissue. Previous studies have shown that matrix metalloproteinases (MMPs) participate in this process [4, 5]. MMPs are a family of zinc-containing peptide hydrolases that can lead to degradation of extracellular matrix proteins (ECMs) [6]. MMP-1, a key member of the MMP family, initiates collagen cleavage and plays a key role in the skin aging process [7]. MMPs expression can be induced by a cascade of phosphorylation of protein [8, 9]. Mitogen-activated

Abbreviations: UV, Ultraviolet; NHDFs, normal human dermal fibroblasts; RI, *Rubus idaeus* L.; MMP-1, matrix metalloproteinase-1; MMP-3, matrix metalloproteinase-3; IL-6, Interleukin-6; MAPK, mitogen-activated protein kinase; AP-1, activator protein 1; TGF-β, transforming growth factor-β; HO-1, heme oxygenase-1; NQ-O1, NHD(P)H quinone oxidoreductase1; Nrf2, nuclear factor E2-related factor 2; ECMs, extracellular matrix proteins; JNK, Jun-N-terminal kinase; ERK, extracellular-regulated protein kinase; p38, p38 mitogen-activated protein kinases; ROS, reactive oxygen species; ARE, antioxidant-response element; DPPH, 2,2-diphenyl-1-picrylhrazyl; DCFH-DA, 2'7'-dichlorofluorescein diacetate; Inhibitory-κβ, I-κβ; Nuclear factor κβ, NF-κβ; Iκβ kinase, IKK

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protein kinases (MAPKs), consisting of Jun-N-terminal kinase (JNK), extracellular-regulated protein kinase (ERK) and p38 kinase, is a well-known ROS-sensitive signal pathway [10]. It's reported that MAPKs phosphorylation could stimulate MMPs gene transcription by the activation of activator protein 1 (AP-1) [11]. Moreover, skin photoaging can also be amplified by UV irradiation-induced inflammation. A previous study found that UV-induced cytokine network consisting of IL-1α, IL-1β and IL-6 in fibroblasts could induce MMP-1 secretion via autocrine loops, resulting in the loss of interstitial collagen [12]. Related report also found the nuclear factor κβ (NF-κβ) activation plays a key role in cytokine-induced MMP-1 expression [13]. Bond *et al* found that NF-κβ had the capacity to induce MMP-1 and MMP-3 upregulation in rabbit dermal fibroblasts [14]. The inactive NF-κβ is commonly localized in cytoplasm binding with a class inhibitory protein known as inhibitory-κβ (I-κβ). It has been proved that UV radiation could activate NF-κβ through Iκβ kinases (IKK)-dependent and IKK-independent Iκβ degradation pathways [15, 16]. The activated NF-κβ causes inflammatory factors, which further stimulate the signal transduction pathway to activate NF-κβ, formed the vicious cycle. Thus, to control these signaling pathways and the expression of inflammatory cytokines might be critical in preventing UV-induced photoaging.

The key mechanism by which UV induces skin aging lies in the upregulation of ROS. Plenty of earlier studies have shown that ROS contribute to UV irradiation-stimulated signal transduction such as MAPK, AP-1 and NF-κβ [17, 18]. Our skin is equipped with various enzymatic and non-enzymatic antioxidant systems that respond to oxidative stress. The NF-E2-related nuclear factor 2 (Nrf2)/antioxidant-response element (ARE) pathway is one of these important cellular defense systems. Upon stimulation, Nrf2 translocates into the nucleus and binds to ARE, which then activates the expression of numerous antioxidant-related genes to maintain cellular redox homeostasis [19]. Ayako *et al.* demonstrated that UVB-irradiated $nrf2^{-/-}$ mice accelerated coarse wrinkle formation, skin flexibility loss, and extracellular matrix deposition, indicating that Nrf2 plays an important role in preventing photoaging [20]. Therefore, elimination of ROS and activation of the Nrf2/ARE pathway are potentially effective strategies in protecting against UVB-induced photoaging.

Beyond the degradation of collagen, UV radiation has been shown to affect procollagen synthesis [21]. Transforming growth factor-β (TGF-β) is a multifunctional cytokine that plays a critical role in regulating procollagen synthesis [22]. TGF-β is reported to initiate its cellular actions by specifically binding to cell-surface receptor, then activates Smad2/3 transcription factor. Phosphorylated Smad2 and Smad3, in association with Smad4, transduces signal to nucleus where subsequently induces the transcription of TGF-β-responsive genes, such as type I procollagen [23, 24]. However, Smad7 is found to be a negative regulator that interferes the activation of Smad2/3 by interacting with TGF-β receptor [25]. It has been reported that UVB radiation impaired TGF-β/Smad pathway through inhibiting the translocation of Smad2/3 to nucleus and up-regulation of Smad7 expression, this impairment is the major inducement for type I procollagen reduction [26].

Rubus idaeus L., also known as red raspberry, is a small fleshy berry that is widely consumed in our diet and has been receiving increasing attention due to its beneficial health effects. The main active components of *Rubus idaeus* L. were shown to be anthocyanins, flavonoids and phenols [27]. Anthocyanins are a class of important antioxidants with extensive pharmacological activities such as reduction of age-associated oxidative stress, and both anti-inflammatory and anticancer effects [28]. Previous studies have reported that *Rubus idaeus* L. (red raspberries) possess excellent antioxidant, anti-inflammatory and antimicrobial activities [29], but there is limited knowledge about its biological activity on skin diseases. Duncan *et al* found that topical treatment with black raspberry could reduce UVB-induced skin inflammation and carcinogenesis [30], but the effect of red raspberry against UVB-induced photoaging had not been investigated. Here, we investigated the anti-aging effects of *Rubus idaeus* L. (RI) extract and the internal mechanism on UVB-irradiated normal human dermal fibroblasts (NHDFs). The results indicated that RI possessed a great potential in reduction of UVB-induced loss of collagen by inhibition of MMPs, inflammatory factors production and upregulation of procollagen synthesis, which was involved in regulation of MAPK/AP-1, NF-κβ, Nrf2 and TGF-β/Smad pathway.

2. Materials and Methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). ELISA kits for MMP-1 and IL-6 were obtained from R&D Systems (R&D Systems, Inc., Minneapolis, MN, USA). Fruit of *Rubus idaeus* L. was purchased from Best Herb corp. (Seoul, Korea). Antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and Cell Signaling Technology (Danvers, MA, USA). Inhibitors of PD98059 and SP600125 were purchased from Cell Signaling Technology (Danvers, MA, USA).

2.2. Sample Preparation

The dried fruit of *Rubus idaeus* L. (50 g) was powdered and extracted three times with 50% ethyl alcohol for 24 h at room temperature. The extracts were then filtered and concentrated with a rotary vacuum evaporation at 40°C. Dried extracts for the cell experiments were dissolved in DMSO. The concentration of DMSO is less than 2‰.

2.3. HPLC Analysis and HPLC/MS Conditions

High performance liquid chromatography (HPLC) was carried out on a Dionex Chromelon TM chromatography data system equipped with P580 and UVD100 detectors (Thermo Fisher Scientific Inc., Waltham, MA USA). The column was a Watcher 120 ODS-AP column (5 μm, 250 × 4.6 mm). Column temperature was set at 25°C. The mobile phases were composed of water with 0.1% formic acid (A) and MeOH, a gradient elution was performed as follows: 0-5 min, 20%B; 5-30 min, 20%-35%B; 30-31 min, 35%-95%B; 31-36 min, 95%B; 36-37 min, 95%-20%B; 37-43 min, 20%B. The flow-rate was 1 mL/min and the detection wavelength was set at 520 nm.

The HPLC/MS analysis was performed on a Waters ACQUITY UPLC system equipped with a Acquity UPLC ®BEH C18 column (1.7 μm, 2.1 × 100 mm), and the column oven was set at 25°C. The separation was conducted using mobile A (0.1% formic acid in acetonitrile) and mobile B (0.1% formic acid aqueous solution) at a flow rate of 0.3 mL/min. The gradient program was set as follows: 0–3 min, 5% B; 3-20 min, 5-28% B; 20-24 min, 28-90% B; 24-25 min, 90-5% B; 25-30 min, 5% B. The wavelength of DAD detector was set at 254nm. Mass detection was performed on a Waters SQ detector via a negative electrospray ionization mode. Nitrogen was used as the drying gas. Desolvation gas flow was 600 L/h, and the cone gas flow was maintained at 50 L/h. Desolvation temperature was 350 °C and source temperature was 150 °C. Observed capillary and cone voltages were 3.0 KV and 45 V, respectively.

2.4. Total Phenolic Content

Total phenolic content was determined according to the adapted Folin–Ciocalteu method in 96 well plates [31]. The extract (50 μL) was mixed with 50 μL of 1N (1:1 diluted stock) Folin–Ciocalteu reagent. After 15 min, 150 μL of 0.7 M Na_2CO_3/NaOH was added to each well and then optical density was measured using VersaMax™ at 765 nm after 1 h incubation. Aqueous solutions of gallic acid were used for calibration. The results are expressed as gram gallic acid equivalents/100 g dry weight (dw).