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Antioxidant effects of the sarsaparilla via scavenging of reactive oxygen species and induction of antioxidant enzymes in human dermal fibroblasts

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ABSTRACT

Ultraviolet (UV) radiation from sunlight causes distinct changes in collagenous skin tissues as a result of the breakdown of collagen, a major component of the extracellular matrix. UV irradiation downregulates reactive oxygen species (ROS)-elimination pathways, thereby promoting the production of ROS, which are implicated in skin aging. *Smilax glabra* Roxb (sarsaparilla) has been used in folk medicine because of its many effects. However, no study on the protective effects of sarsaparilla root (SR) on human dermal fibroblasts has been reported previously. Here, we investigated the protective effect of SR against oxidative stress in dermal fibroblasts. SR significantly inhibited oxidative damage and skin-aging factor via mitogen-activated protein kinase signaling pathways. Also, SR decreased Ca^{2+} and ROS, mitochondrial membrane potential, dysfunction, and increased glutathione, NAD(P)H dehydrogenase and heme oxygenase-1. These results demonstrate that SR can protect dermal fibroblasts against UVB-induced skin aging via antioxidant effects.

1. Introduction

Skin aging can be attributed to extrinsic and intrinsic processes that commonly manifests as increased wrinkling, sagging, and laxity (Ganceviciene et al., 2012). Ultraviolet (UV) radiation from sunlight, particularly UVB with wavelengths of 290–320 nm, causes distinct changes in collagenous skin tissues as a result of the breakdown of collagen, a major component of the extracellular matrix (Ganceviciene et al., 2012). Exposure of skin to UVB induces the intracellular and

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extracellular generation of large quantities of calcium (Ca^{2+}) and reactive oxygen species (ROS) (Bito and Nishigori, 2012; Masaki et al., 2009; Lyu and Park, 2012). Ca^{2+}- and ROS-induced molecular damage produces a number of harmful effects on cellular function and homeostasis, while degradation of extracellular matrix proteins, such as collagen, by Ca^{2+} and ROS can cause major changes in skin connective tissue (Masaki et al., 2009). In fact, UV-induced Ca^{2+} and ROS can lead to autoimmune disease and inflammation in the skin, as well as skin cancer, while induction of matrix metalloproteinase (MMP) expression can cause collagen degradation (Kato et al., 2010). These alterations in the extracellular matrix, most likely mediated by MMPs, are known to be a cause of the skin wrinkling that characterizes premature skin aging.

Smilax glabra Roxb, a member of the Liliaceae family, shares the name sarsaparilla with other species of Smilax around the world (including *S. officinalis*, *S. aristolochiaefolia*, *S. febrifuga*, *S. regelii*, *and S. japicanga*) (Ooi et al., 2004). The leaves and small black fruit of sarsaparilla have a liquorice-like, sweet taste. Generally, the plant is used as a substitute for tea and sugar, to prevent scurvy, and for treating a range of different conditions, including chest ailments, rheumatism, leprosy, impotence, and syphilis (Ooi et al., 2004; Sa et al., 2008; Das and Bisht, 2013). Syrup made by prolonged boiling of the leaves was marketed in Sydney in the early 1900s as a tonic and a remedy against catarrh and coughs (Cox et al., 2005). In addition, the roots have been used in the West as an ingredient in root beer and other beverages because of their foaming properties (Cox et al., 2005). *S. glabra* (Chinese name *S. glabra* 'tufuling') is a very important herbal medicine. Its root has been used in folk medicine because of its many effects, such as anti-inflammatory, anti-venereal, and detoxifying activities (Ooi et al., 2008; Xia et al., 2010). Although antioxidative effects of sarsaparilla have been demonstrated, the effect on skin aging has not been examined.

Here, we investigated the protective effects of sarsaparilla against UVB- or H_2O_2-induced oxidative damage in human dermal fibroblasts. Additionally, we aimed to develop better the mechanisms of action of sarsaparilla by measuring radical scavenging activities, levels of extracellular Ca^{2+}, intracellular ROS, total glutathione (GSH), NAD(P)H dehydrogenase (NQO1), and heme oxygenase-1 (HO-1), mitochondrial dysfunction, and levels of skin aging factors such as MMP-1 and collagen type I alpha 1 (COL1A1) proteins.

2. Materials and methods

2.1. Chemical

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and 2,7-dichlo rodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma–Aldrich (St. Louis, USA). Rabbit anti-HO-1 was obtained from assay designs (Ann Arbor, USA). Mouse anti-β-actin was obtained from Santa Cruz (Santa Cruz, CA). Mouse anti-COL1A1 was obtained from Abnova (Golden, CO). Goat anti-NQO1 was obtained from Abcam (Cambridge, UK). Rabbit anti-extracellular signal-regulated kinases (ERK), phospho-ERK (pERK), p38 mitogen-activated protein kinases (p38), phospho-p38 (pp38), c-Jun N-terminal kinases (JNK), and phospho-JNK (pJNK) were obtained from Cell Signaling (Beverly, MA). The other reagents used were of analytical grade.

2.2. Preparation of the sarsaparilla extract

Root of sarsaparilla (SR) was purchased from Starwest Botanicals, Inc. (Rancho Cordova, CA) and a voucher specimen (KHUOPS-GCH002) was deposited in the herbarium at the College of Pharmacy, Kyung Hee University (Seoul, Korea). 100 g of SR were ground with 1 L of 70% Et-OH for 24 h at room temperature. Then, the extract was filtered, evaporated on a rotary vacuum evaporator, and lyophilized (yield; 9.12%). The powder (SRE) was kept at 4 °C before use.

2.3. Cell culture

The HS68 cell line, human dermal fibroblast cell, was obtained from the American Type Culture Collection (ATCC; Rockville, USA). Cells were maintained in DMEM supplemented with 10% heat inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin in condition of 95% air and 5% CO_2 at 37 °C. All experiments were carried out 12 h after cells had been seeded in 96-well plates for cytotoxicity, NADH dehydrogenase assay and 24-well plates for kit assay, and 100 mm dish for Western blotting assay, at densities of 1×10^5 cells/mL and 2×10^5 cells/mL, respectively. Also, the hemocytometer is a device for counting cells.

2.4. UVB irradiation

The cells were rinsed twice with phosphate-buffered saline (PBS), and all irradiations were performed under a thin layer of PBS. The plate was closed during irradiation. UVB radiation was supplied by a closely spaced array of 5 Sankyo Denki sunlamps, which delivered uniform radiation at a distance of 7.5 cm. The cells were irradiated with UVB (50 mJ/cm^2) for 40 s. Immediately after irradiation, the cells were washed 3 times with warm PBS, after which 198 μL of fresh serum-free medium and 2 μL of sample were added to each well for the indicated time. Control cells were kept in the same culture conditions without UVB exposure.

2.5. Measurement of cytotoxicity

Release of lactate dehydrogenase (LDH) was determined using a CytoScanTM LDH-cytotoxicity assay kit, according to the instruction manual. Briefly, the supernatants (100 μL) were centrifuged, transferred, and reacted with 100 μL of the mixture of dye solution in the dark for 30 min. Absorbance at 490 nm was measured.

2.6. Measurement of intracellular Ca^{2+}

Intracellular calcium concentration was measured using Fluo-4 NW calcium assay kit. The membrane-permeable Fluo-4 AM, a fluorescent calcium indicator, is converted to Fluo-4 within the cell, and calcium binding increases the green fluorescence of Fluo-4. Cells were seeded in a 96-well plate at a density of 1.0×10^5 cells/well. Then, treated with green tea