Sauchinone, a Lignan from *Saururus chinensis*, Protects Human Skin Keratinocytes against Ultraviolet B-Induced Photoaging by Regulating the Oxidative Defense System

Gunhyuk Park, a Hyo Geun Kim, b Yeomoon Sim, b Sang Hyun Sung, c and Myung Sook Oh* a,b

*Department of Life and Nanopharmaceutical Science and Kyung Hee East-West Pharmaceutical Research Institute, Kyung Hee University; b Department of Oriental Pharmaceutical Sciences, College of Pharmacy, Kyung Hee University; 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 130–701, Republic of Korea; and * Institute for Life Science, Elocscience Co., Ltd., and College of Pharmacy, Seoul National University; 56–1 San, Sillim-dong, Gwanak-gu, Seoul 151–742, Republic of Korea.

Received February 1, 2013; accepted April 29, 2013

© 2013 The Pharmaceutical Society of Japan

The authors declare no conflict of interest.

*To whom correspondence should be addressed. e-mail: msohok@khu.ac.kr

Skin aging can be attributed to intrinsic and extrinsic aging (photoaging).1) Damage to skin due to passage of time (intrinsic aging) and to repeated exposures to ultraviolet (UV) radiation (photoaging) are considered distinct entities.1) UV radiation, particularly UVB (290–320 nm) from sunlight, leads to direct DNA damage, reactive oxygen species (ROS) formation, and the associated to the extracellular matrix structural integrity.2) UVB-irradiation induces the over-expression of various proteins that are involved in cellular damage responses and signaling-transduction pathways.2,3) Particularly, the expression of UVB-induced matrix metalloproteinase-1 (MMP-1), the interstitial collagenase, leads to the breakdown of collagen and is thus related to photoaging.4) The most abundant structural protein in skin connective tissue is type 1 collagen (COL1A1), which is responsible for conferring resiliency and strength.5,5) Primarily, skin aging is related to reduction in COL1A1 levels, which is the principal component of skin.

Sauchinone is lignan isolated from *Saururus chinensis* that has been used in Oriental medicine for the treatment of several inflammatory diseases including edema and jaundice.7) *Saururus chinensis* has been reported to have various bioactivities including anti-inflammatory, hepatoprotective, and antioxidant effects.6–8) Moreover, sauchinone has been known to inhibit apoptotic damage of glia cells via suppression of free radical production.7) Additionally, sauchinone inhibits lipopolysaccharide-induced expression of tumor necrosis factor-α level via activation of nuclear factor-kappa B.8) Although inflammatory and antioxidant effects of sauchinone have been demonstrated, the effect on photoaging has not been examined.

Ultraviolet (UV) radiation from sunlight induces matrix metalloproteinase (MMP) expression, which are responsible for collagenous extracellular matrix proteins breakdown in skin, causing photoaging. Sauchinone is reported to have various bioactivity such as antioxidative, hepatoprotective, and anti-inflammatory effects. In the present study, we investigated the protective effect of sauchinone against UVB (50 μJ/cm²)-induced pho-toaging in HaCaT human epidermal keratinocytes. Sauchinone, at 5–40 μM, significantly protected keratinocytes against UVB-induced damage as assessed by cell viability and toxicity assay. Additionally, sauchinone, at 20–40 μM, prevented the upregulation of MMP-1 proteins and reduction of type 1 collagen induced by UVB. Other assays revealed that, in keratinocytes, sauchinone decreased reactive oxygen species (ROS) production and increased glutathione levels and heme oxygenase-1. Sauchinone also inhibited UVB-induced phosphorylation of mitogen-activated protein kinase (MAPK) signaling pathways. These results demonstrated that sauchinone protects skin keratinocytes through inhibition of extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 MAPKs signaling via upregulation of oxidative defense enzymes.

Key words: sauchinone; matrix metalloproteinase-1; antiphotoaging; antioxidative; type 1 collagen; ultraviolet B
penicillin, and $100 \mu g/mL$ 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 ninety-six-well and twenty-four plates, at densities of $1 \times 10^4$ cells/well and $2 \times 10^4$ cells/well, respectively.

**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 $\mu L$ of fresh serum-free medium and 2 $\mu L$ of sample were added to each well for the indicated time. Control cells were kept in the same culture conditions without UVB exposure.

**Measuring Cell Viability** After cells were seeded on 96-well plates, they were treated with sauchinone at doses of 5, 10, 20, and $40 \mu M$ or for 23 h or followed by stimulation with 50 mJ/cm$^2$ of UVB for an additional 23 h. Then, treated cells were incubated with 1 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 2 h. The MTT solution was aspirated from the wells and the dark blue formazan crystals was eluted using DMSO. The absorbance was measured using a spectrophotometer (Versamax microplate reader; Molecular Device, Sunnyvale, CA, U.S.A.) at a wavelength of 570 nm.

**Measuring Intracellular ROS** The production of intracellular ROS was measured using fluorescence dye DCFH-DA. DCFH-DA is converted into non-fluorescent DCFH in cells, which reacts with ROS to form the DCF fluorescence. HaCaT cells were treated with sauchinone at doses of 10, 20, and $40 \mu M$ for 1 h. Then, they were stimulated with 25 $\mu M$ DCFH-DA for 30 min. Representative images and fluorescence intensity were taken using a fluorescence microscope (Olympus Microscope System BX51; Olympus, Tokyo, Japan).

**Measuring the MAPKs, HO-1, and COL1A1 Levels** The cells were seeded on a 100-mm dish and treated with sauchinone at doses of 20 and $40 \mu M$ for 1 h. Then, they were stimulated with 50 mJ/cm$^2$ for an additional 1 or 23 h. The lysates were separated on 15 or 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then proteins were transferred to a membrane. The membranes were incubated with 5% skim milk in TBST and primary antibody (1:500–2000 dilution) overnight at 4°C, followed by incubation with HRP-conjugated secondary antibody immunoglobulin G for 1 h, respectively. Immunoreactive bands were detected and visualized using enhanced chemiluminescent direct labeling detection kit and LAS-4000 mini system (FUJIFILM Corp., Japan), respectively.

**Measuring the Total GSH Level** Total GSH was determined using a total glutathione quantification kit (Dojindo Molecular Technologies, Japan) according to the manufacturer’s directions.

**Measuring the MMP-1 Level** MMP-1 was measured using a Human MMP-1 ELISA kit (RayBiotech, U.S.A.) according to the manufacturer’s directions.

**Statistical Analysis** All statistical parameters were calculated using Graphpad Prism 4.0 software. Values were expressed as the mean±standard error of the mean (S.E.M.) and statistical comparisons were performed using one-way ANOVA analysis. Differences with a $p$-value less than 0.05 were considered statistically significant.

**RESULTS**

**Protective Effects of Sauchinone against UVB-Induced Damage in HaCaT Human Epidermal Keratinocytes** To evaluate the protective effects of sauchinone against UVB-induced toxicity in HaCaT cells, we measured cell viability using MTT assay. Treatment with 5–40 $\mu M$ sauchinone or $100 \mu M$ vitamin C alone had no effect on the cells (Fig. 2A).
Treatment with 50 mJ/cm² of UVB for an additional 23 h reduced cell viability to 58.80±4.41%, as compared to control cells. Pre-treatment with sauchinone limited this reduction in cell viability. Cell viability in cells pre-treated with 5–40 µM sauchinone was 67.84±1.58 to 90.93±7.03% of control values (Fig. 2B). In this regard, 100 µM vitamin C had an effect similar to those of 20 µM (positive controls).

Protective Effects of Sauchinone against UVB-Induced Levels of MMP-1 and Collagen Type I Proteins To investigate the antiphotoaging effects of sauchinone on UVB-induced damage, we measured cellular COL1A1 levels and MMP-1 secretion. UVB caused significant elevation of MMP-1 and depletion of COL1A1 (by 216.14±22.05 and 57.82±3.57%, respectively), while sauchinone decreased levels of MMP-1 and increased levels of COL1A1 (by 186.89±1.53 to 151.67±10.13% and 85.56±2.06 to 110.49±13.95%, respectively) (Fig. 3).

Protective Effects of Sauchinone against UVB-Induced Intracellular ROS Generation To examine whether sauchinone affected toxicity is related to antioxidant effects, we assessed intracellular ROS generation using DCFH-DA. Treatment with UVB significantly increased ROS generation compared with control cells (by 215.70±14.38%), while treatment with 10, 20, and 40 µM sauchinone reduced UVB-induced ROS generation (by 126.25±13.21 to 101.94±13.05%, respectively) (Fig. 4).

Protective Effects of Sauchinone against UVB-Induced Oxidative Defense Enzyme Such as HO-1, and GSH Proteins To examine whether sauchinone affected the oxidative defense enzyme, we measured levels of antioxidative-related enzyme such as HO-1 and total GSH levels. UVB caused significant HO-1 and GSH depletion (by 60.39±6.37 and 58.01±
1.03%, respectively), while sauchinone at 20–40 µM increased HO-1 and GSH levels (by 70.96±8.36 to 98.60±11.05% and 79.56±0.63 to 93.09±2.30%, respectively) (Figs. 5A, B).

**Protective Effects of Sauchinone against UVB-Induced MAPKs Proteins** To examine whether sauchinone affected the MAPKs signaling pathways, we measured levels of ERK, JNK, p38 protein. UVB caused significant activation of pERK, pJNK, and pp38 protein (by 536.90±66.14, 3038.95±192.43, and 2852.78±322.63%, respectively), while sauchinone at 20–40 µM decreased levels of pERK, pJNK, and pp38 protein (by 414.51±24.21 to 190.75±26.67%, 2170.97±127.59 to 1127.20±106.21%, and 2110.58±334.46 to 1314.85±163.21%, respectively) (Fig. 6).

**DISCUSSION**

In the current study, we demonstrated that sauchinone prevents or inhibits UVB-induced expression of COL1A1 or MMP-1 through inhibition of ERK, JNK, and p38 MAPK signaling pathways and expression of antioxidative-related enzymes.

First, to investigate the protective and anti-photoaging effect of sauchinone on UVB-induced cell toxicity, we measured cell viability, cellular COL1A1 levels, and MMP-1 secretion. Accordingly, UV-induced MMP-1 expression induces the cleavage of collagen fibers. Once collagen is cleaved by MMP-1, collagen degradation is further promoted by various MMPs. MMP-1, a fibroblast-type or interstitial collagenase, is secreted by keratinocytes, fibroblasts, and macrophages. These properties make MMP-1 an attractive target for the pharmacological development of anti-photoaging agents. In the present study, sauchinone significantly protected against 50 mJ/cm² UVB-induced damage at 10–40 µM without inducing toxicity in human epidermal keratinocyte cells (Fig. 2). Additionally, UVB caused significant elevation of MMP-1 and depletion of COL1A1, whereas sauchinone attenuated MMP-1 and COL1A1 levels (Fig. 3). These results indicate that sauchinone prevents UVB-induced collagen damage through elevation of MMP-1 and reduction of COL1A1, which are related to anti-photoaging.

Next, to examine whether the protective effect of sauchinone against UVB-induced cell toxicity is age related to antioxidative effects, we assessed intracellular ROS generation using DCFH-DA. Current studies, UV exposure in the skin is associated with the UV irradiation-induced generation of reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide, hydroxyl radical and singlet oxygen as...
well as lipid peroxides and their radicals.13) ROS are potential inducers of MMP-1 protein and cause intracellular oxidative damage in skin.13–15) In the present study, sauchinone largely inhibited the overproduction of intracellular ROS induced by UVB (Fig. 4). These results indicate that sauchinone protected against photaging from UVB-induced ROS. Additionally, to examine whether sauchinone affected the oxidative-regulatory system, we measured levels of oxidative defense enzymes such as HO-1 and GSH protein, which repair skin by reducing the photaging by the most common free radical.15,16) HO-1 is the rate-limiting enzyme in the catalabolism of heme, which results in the release of carbon monoxide, iron, and biliverdin.13) Also, glutathione is involved in both non-enzymatic and enzymatic defences against ROS.13,16) In addition to its role as a free-radical scavenger in cells, it is a co-factor for several enzymes involved in the antioxidant defense mechanism.16)

These include glutathione peroxidases, which reduce H₂O₂ and organic peroxides using GSH, and glutathione reductase, which re-cycles oxidised glutathione to its reduced form. In the present study, UVB caused significant HO-1 and GSH depletion, whereas sauchinone increased HO-1 and GSH levels (Fig. 5). Also, we usually used to evaluate radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, sauchinone has shown very lower activity in DPPH compared with the vitamin C known to be a potential anti-oxidant.17)

Taken together, these results indicate that sauchinone weak radical-scavenging antioxidants but can be potent inhibition of the ROS levels via up-regulating HO-1 and GSH levels induced by UVB (data not shown).

Finally, because MAPK-dependent pathways are involved in MMP-1 secretion via ROS generation, we measured ERK, JNK, and p38 protein levels. Phosphorylated MAPKs signal cellular functions, including MMP-1 expression in skin cells.18) From the bark and seed cones of nature plants such as sauchinone have been suggested to play antioxidative-related enzymes. Recently, lignan compounds from plants such as sauchinone have been suggested to play a role in protection via antioxidant effects in skin.16,19) For example, macelignan isolated from *Myristica fragrans*, gomisin C isolated from *Schizandra chinensis*, and magnolol isolated from the bark and seed cones of *Magnolia officinalis* show the most potent protective effects to date.17,20) Furthermore, studies have revealed that sauchinone reduced the release of superoxide dismutase and glutathione peroxidase from cheekbone ligand 4 damaged in hepatocytes.19) Sauchinone also seemed to ameliorate lipid hydroperoxide, as demonstrated by a reduction in the production of malondialdehyde.8,21) While much previous research on sauchinone has focused on anti-inflammatory and antioxidant effects in hepatocytes, less is known regarding skin disease. We first demonstrated that sauchinone protected keratinocytes, illustrated by protection against UVB-induced photoaging, by inhibiting MAPK signaling pathways via ROS generation. Taken together, signaling factors may contribute, at least in part, to the protective effects of sauchinone against UVB damage, although this remains to be further studied.

CONCLUSION

Our study demonstrates that sauchinone inhibits UVB-induced MMP-1 expression, which is mediated by inhibition of ERK, JNK and p38 MAPKs signaling via antioxidant effects in HaCaT human epidermal keratinocytes. Our data suggest that sauchinone is a potential agent for the management of skin photoaging.

Acknowledgement This study was supported by a Grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (F110021).

REFERENCES


