INTRODUCTION

Prostaglandins (PGs) are members of the eicosanoid family and are well-known to have various normal and pathophysiological responses, such as vessel contraction/relaxation, renal filtration, angiogenesis, increased proliferation, and immune suppression (Harris et al., 2002; Flower, 2006). PGE$_2$ is the most abundant PG in the human body and synthesized from arachidonic acid via the actions of cyclooxygenase (COX) enzymes. PGE$_2$ exerts homeostatic, cytoprotective, inflammatory, and in some cases anti-inflammatory effects. Also, it has been reported that PGE$_2$ is involved in hair growth. Diphlorethohydroxycarmalol (DPHC) is a phlorotannin compound isolated from the brown algae *Ishige okamurae*, with various biological activities in vitro and in vivo. In this study, the biological effect and mechanism of action of DPHC on prostaglandin synthesis in HaCaT human keratinocytes was examined. The results showed that, in these cells, DPHC significantly and dose-dependently induced PGE$_2$ synthesis by increasing the protein and mRNA levels of COX-1 and COX-2. Interestingly, DPHC-induced COX-1 expression preceded that of COX-2. Also, while both rofecoxib and indomethacin inhibited PGE$_2$ production, the latter was seems to be the more potent. From above results, we can expect that DPHC has some beneficial effects via increasing of PGE$_2$ production.

Key Words: Diphlorethohydroxycarmalol, Cyclooxygenases, Prostaglandin E$_2$, HaCaT keratinocytes

Diphlorethohydroxycarmalol, Isolated from *Ishige okamurae*, Increases Prostaglandin E$_2$ through the Expression of Cyclooxygenase-1 and -2 in HaCaT Human Keratinocytes

Gyeoung-Jin Kang$^1$, Sang-Chul Han$^1$, Young-Sang Koh$^2$, Hee-Kyoung Kang$^1$, You-Jin Jeon$^3$ and Eun-Sook Yoo$^1$,*

Departments of $^1$Pharmacology, $^2$Microbiology, School of Medicine, Jeju National University, Jeju 690-756, $^3$Aqua Green Technology Co., Jeju Bio-Industry Center, Jeju 690-121, Republic of Korea

Abstract

Prostaglandin (PG) E$_2$, the most abundant prostaglandin in the human body, is synthesized from arachidonic acid via the actions of cyclooxygenase (COX) enzymes. PGE$_2$ exerts homeostatic, cytoprotective, inflammatory, and in some cases anti-inflammatory effects. Also, it has been reported that PGE$_2$ is involved in hair growth. Diphlorethohydroxycarmalol (DPHC) is a phlorotannin compound isolated from the brown algae *Ishige okamurae*, with various biological activities in vitro and in vivo. In this study, the biological effect and mechanism of action of DPHC on prostaglandin synthesis in HaCaT human keratinocytes was examined. The results showed that, in these cells, DPHC significantly and dose-dependently induced PGE$_2$ synthesis by increasing the protein and mRNA levels of COX-1 and COX-2. Interestingly, DPHC-induced COX-1 expression preceded that of COX-2. Also, while both rofecoxib and indomethacin inhibited PGE$_2$ production, the latter was seems to be the more potent. From above results, we can expect that DPHC has some beneficial effects via increasing of PGE$_2$ production.

Key Words: Diphlorethohydroxycarmalol, Cyclooxygenases, Prostaglandin E$_2$, HaCaT keratinocytes

INTRODUCTION

Prostaglandins (PGs) are members of the eicosanoid family and are well-known to have various normal and pathophysiological responses, such as vessel contraction/relaxation, renal filtration, angiogenesis, increased proliferation, and immune suppression (Harris et al., 2002; Flower, 2006). PGE$_2$ is the most abundant PG in the human body and synthesized from arachidonic acid via the actions of cyclooxygenase (COX) enzymes. PGE$_2$ exerts homeostatic, cytoprotective effects, inflammatory, and in some cases anti-inflammatory effects (Giuliano and Warner, 2002; Ricciotti and FitzGerald, 2011). COX enzymes comprise two isofoms, COX-1 and COX-2. COX-1 is constitutively expressed and its physiological functions include roles in the maintenance of vascular flow, cell division, mucus and bicarbonate production, and several cellular “housekeeping activities.” Its counterpart, COX-2, is an inducible enzyme with specialized functions, including the setting of inflammation, fever, pain, and stress adaptation (Langenbach et al., 1999; Tanabe and Tohnai, 2002).

Recently, it has been reported that it is the relationship between hair regrowth and PGE$_2$. In previous studies, PGE$_2$ showed moderate growth stimulatory effects on early anagen hair follicles in mouse and PG analog, latanoprost, stimulated eyelashes growth in human (Johnstone and Albert, 2002; Sasaki et al., 2005). Also, Hoggaat et al. (2009) reported that PGE$_2$ may stimulate the survival and proliferation of hematopoietic stem cells. In 1990’s, there are reports that minoxidil (MXD), used as a hair loss therapeutics, stimulated the activity of purified ovine prostaglandin G/H synthase (PGHS-1; COX-1) in vitro and increased the production of PGE$_2$ in cultured human or rat dermal papilla cells, and mouse fibroblasts (Michellet et al., 1997; Lachgar et al., 1998).

Marine algae, such as Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae), produce a wide variety of structurally unique and biologically active secondary metabolites. These compounds have been the focus of recent intense interest due to their potential as bioactive ingredients in cosmetics and as therapeutic agents. Among the latter, alpha-kainic acid, domoic acid, and bryostatin-1, isolated from marine organisms, have been used as cytotoxic agents. *Ishige okamurae*, a member of the Ishigeaceae family, is an edible brown alga found along the coast of Jeju Island, Korea. It is the source of diphlorethohydroxy-
carmalol (DPHC), a biologically active phlorotannin. For example, DPHC was shown to alleviate postprandial hyperglycemia in diabetic mice (Heo et al., 2009) and to protect cells from UV-B radiation-induced damage (Heo et al., 2010). In a recent report, DPHC inhibited melanogenesis and protected mice against radiation-induced cell damage (Ahn et al., 2011). In 2009, Kim et al. reported the anti-inflammatory properties of an ethanolic extract of I. okamurae whose activities included the inhibition of nuclear factor kappa B (NF-κB).

Keratinocytes are most important in epidermis of skin and a hair follicle producing hair is occurred from the stratum basale of the epidermis and is located in the dermis. Therefore, in the present study, we investigated that a relative effect of DPHC on the production of PGE$_2$ when compared with MXD and that a mechanism of action by examining the expression of COX-1 and COX-2 in human HaCaT keratinocytes.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Fetal bovine serum (FBS) and Dulbecco’s modified Eagle’s medium (DMEM) were purchased from Invitrogen (Grand Island, NY, USA). COX-1, COX-2, PGDH, and the GAPDH primers for end-point PCR were obtained from Cosmo Gene Tech (Korea), and anti-COX-1 and anti-COX-2 from Cell Signaling Technology (Beverly, MA, USA). DyLight488-conjugated donkey anti-rabbit antibody was purchased from BioLegend (San Diego, CA, USA). All other chemicals were of reagent grade.

**DPHC isolation**

Diphlorethohydroxycarmalol (DPHC) was isolated as previously described (Heo et al., 2008). Briefly, dried I. okamurae was extracted three times with 80% methanol and then filtered. The filtrate was suspended in distilled water and partitioned with ethyl acetate. The resulting fraction was subjected to silica gel and Sephadex-LH 20 column chromatography. DPHC was finally purified by high performance liquid chromatography (HPLC), and its structure (Fig. 1A) was confirmed by comparing the NMR spectral data with those in the existing literature.

**Cell culture and cell viability**

HaCaT human keratinocytes were cultured in DMEM supplemented with 10% heat-inactivated FBS, streptomycin (100 μg/ml), and penicillin (100 U/ml) at 37°C in a humidified CO$_2$ incubator. Cell viability was determined by the WST-1 assay. Cells were seeded in 96-well plates and treated as described in the Results for 24 hr. WST solution was then added to each well, followed by a further incubation for 4 hr. The absorbance of each well at 450 nm was then measured in a VersaMax ELISA microplate reader (Molecular Devices, CA, USA).

**PGE$_2$ analysis by ELISA**

The concentration of PGE$_2$ in the supernatant was measured with an ELISA kit according to the manufacturer’s instructions. Cells in 96-well plates were treated with DPHC or minoxidil for 24 hr. PGE$_2$ standards or cell culture supernatants were then added to the appropriate wells together with a PGE$_2$ alkaline phosphatase tracer. The samples were incubated for 2 hr at room temperature (RT) on an orbital shaker, washed, and then treated by the addition of para-nitrophosphatase (pNPP) to each well. The plates were further incubated for 1 hr at RT in the dark, after which the optical density of each well, determined at 405 nm, was measured using a VersaMax ELISA microplate reader.

**Reverse-transcriptase polymerase chain reaction (RT-PCR)**

Total RNA was isolated from HaCaT cells using the TRI reagent (MRC) according to the manufacturer’s instructions and stored at −70°C until use. Reverse transcription was performed using a First-Standard CDNA synthesis kit (Promega). Total RNA (1 μg) was incubated with an oligo(dT)$_n$ primer at 70°C for 5 min and the reactions then cooled on ice for 5 min. After addition of the reverse-transcriptase premix, the reactions were incubated at 42°C for 60 min and then terminated by incubation at 70°C for 15 min. PCR was conducted using i-Taq™ DNA polymerase (iNTRON Biotechnology, Korea) with the appropriate sense and antisense primers for COX-1, COX-2, PGDH, and GAPDH. The primer sequences were as follows: COX-1: (F) 5'-AAG TAC CAG GTG CGT GAT GG-3', (R) 5'-GCT GCA GGA AAT AGC CAC TC-3' (319 bp); COX-2: (F) 5'-CCA CCC GCA GTA CAG AAA GT-3', (R) 5'-CAG GAT ACA GCT CCA CAG CA-3' (196 bp); PGDH: (F) 5'-GCT GAC GAC ACA CAA CTG AG-3', (R) 5'-GGG CAT CAG TCC TGC TAA AG-3' (237 bp); and GAP-

![Fig. 1. Effect of DPHC on PGE$_2$ production in HaCaT human keratinocytes. (A) Chemical structure of diphlorethohydroxycarmalol (DPHC). (B) Cells (2.0×10$^5$ cells/ml) were cultured for 18 hr and then treated with DPHC (12.5, 25, 50 μg/ml) for 24 hr. Cell viability was then determined in a WST assay. (C) Cells were cultured for 18 hr and then treated with minoxidil (MXD, 100 μM) and DPHC (12.5, 25, 50 μg/ml) for 24 hr. PGE$_2$ levels were determined in an ELISA using the cell supernatants in triplicate samples. Error bars indicate ± S.D. *p<0.05, significant compared to the negative control.](www.biomolther.org)
DH: (F) 5'-GAG TCA ACG GAT TTG GTC GT-3', (R) 5'-GAC AAG CTT CCC GTT CTC AG-3' (185 bp). PCR was performed using a C1000 instrument (Bio-Rad, Hercules, CA, USA) and the following thermal cycling conditions: 94°C for 30 sec, annealing at 55-60°C for 30 sec, and extension at 72°C for 2 min, repeated 30-35 times, followed by incubation at 72°C for 10 min. The reaction products were visualized by electrophoresis on a 1.2% agarose gel (Promega, Madison, WI, USA) and UV light illumination after staining with ethidium bromide. Relative intensities were analyzed using the Image J program.

SDS-PAGE and Western blot analysis

The incubated cells were washed twice with ice-cold PBS and then disrupted in lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Nonident P-40, 2 mM EDTA, 1 mM EGTA, 1 mM NaVO₄, 10 mM NaF, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 25 µg leupeptin/ml) on ice for 30 min. The resulting cell lysates were centrifuged at 15,000 rpm for 15 min at 4°C; the supernatants were used for Western blotting. The total protein concentration of each sample was quantified by the Bio-Rad assay (Bio-Rad). Extracts containing 30 μg of protein were loaded alongside a pre-stained protein molecular mass ladder (Bio-Rad) on a NuPAGE 4-12% bis-Tris gel (Invitrogen, Carlsbad, CA, USA). The proteins were electroblotted onto a polyvinylidene difluoride (PVDF) membrane using an iBlot gel transfer device (Invitrogen). The membrane was blocked with blocking buffer (5% skim milk in TTBS buffer: Tween 20-Tris-buffered saline) for 1 hr at RT, followed by incubation with primary antibodies (1:1,000) overnight at 4°C. All antibodies were diluted in 1% BSA in TTBS buffer. The membrane was washed, incubated with horseradish peroxidase (HRP)-conjugated anti-primary host IgG antibody (diluted 1:5,000) for 1 hr at RT, and then washed again. Immunoreactive bands were visualized with a Western blot detection system (iNtRON Biotechnology, Korea) according to the manufacturer’s instructions.

Confocal microscopy analysis

The cells were seeded onto coverslips in a 6-well plate, fixed with freshly prepared 3.5% paraformaldehyde for 30 min, and permeabilized with 0.1% Triton X-100 for 10 min. After 1 hr incubation with 3% BSA/0.1% Triton X-100/PBS, the cells were treated with primary anti-COX-1 (1:200) or anti-COX-2 (1:200) antibody overnight at 4°C. The cells were washed and then incubated with DyLight488-conjugated donkey anti-rabbit secondary antibody (1:300) for 30 min at RT. After sev-
eral additional washing steps, the coverslips were mounted in VECTASHIELD mounting media with DAPI (Vector Labs, Burlingame, CA, USA). The results were visualized using FV500 confocal microscopy (Olympus, Japan).

Statistical analysis
Student’s t-test and a two-way analysis of variance (ANOVA) were used to determine the statistical significance of differences between experimental and control group values. Data represent the mean ± the standard deviation.

RESULTS

Effect of DPHC on the viability of HaCaT keratinocytes
To evaluate the effect of DPHC on cell viability, WST assays were performed. HaCaT keratinocytes were treated with DPHC at the concentrations indicated in Fig. 1B. As seen in the figure, concentrations below 50 μg DPHC/ml had no effect on cell viability in HaCaT keratinocytes.

Effect of DPHC on PGE\(_2\) production
MXD has been well known to produce PGE\(_2\). Therefore, the effects of MXD and DPHC on PGE\(_2\) production in HaCaT keratinocytes were determined by analyzing culture supernatants in cells treated with these compounds for 24 hr. The results showed that MXD (100 μM) increased PGE\(_2\) production (875.3 ± 41.1 pg/ml), while for DPHC, the increase in PGE\(_2\) was dose-dependent (365.2 ± 47.6, 830.0 ± 39.0, and 1013.2 ± 75.0 pg PGE\(_2\)/ml at 12.5, 25, and 50 μg DPHC/ml, respectively, Fig. 1C).

Effect of DPHC on COX-1 and COX-2 protein expression
The effect of DPHC on the COX enzymes was confirmed at the protein level in HaCaT keratinocytes. Western blot and immunofluorescence analyses showed that DPHC treatment significantly increased the protein levels of COX-1 and COX-2. Specifically, DPHC treatment induced the expression of COX-1 proteins after 6 hr, with a steady increase for up to 24 hr. By contrast, the DPHC-mediated induction of COX-2 protein began after 12 hr and was weaker than that of COX-1. Moreover, a higher concentration of DPHC was needed to increase COX-2 expression (Fig. 2A, B). As seen in the time profile (Fig. 2C), DPHC induced COX-1 expression at an early phase (at 3 hr), while COX-2 was delayed.

Effect of DPHC on the expression of COX-1, COX-2, and 15-PGDH mRNA
A time course of COX-1 and COX-2 mRNA expression was obtained by measuring the mRNA levels of both enzymes in HaCaT keratinocytes treated with DPHC for 3, 6, 12, and 24 hr. COX-1 mRNA expression was induced after 3 hr of DPHC treatment, with a further increase up to 6 hr, followed by a decrease beginning at 12 hr. The COX-2 expression profile differed, with a slight increase up to 24 hr (Fig. 3A, B). These mRNA expression profiles of COX-1 and -2 correlated with the intensity of the respective protein bands in the time profile (Fig. 2C).

15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is a key prostaglandin catabolic enzyme, and its cellular inhibition leads to the accumulation of PGE\(_2\) (Tai et al., 2011). Therefore, we asked whether the DPHC-mediated increase in COX-1 and COX-2 involved the inhibition of 15-PGDH. The mRNA levels of this enzyme were therefore measured in cells treated with DPHC. As shown by RT-PCR, incubation of the cells with 50 μg DPHC/ml for as long as 24 hr had no effect on 15-PGDH mRNA levels (Fig. 3C). These results suggested that DPHC increases PGE\(_2\) expression via the regulation of prostaglandin synthases (COX-1 and COX-2), rather than 15-PGDH.

Fig. 3. Effect of DPHC on COX-1, COX-2, and PGDH mRNA expression in HaCaT human keratinocytes. Cells were cultured for 18 hr and then treated with DPHC (50 μg/ml) for the indicated times. COX-1 (A), COX-2 (B), PGDH (C), and GAPDH mRNA expression was determined by reverse-transcriptase PCR.
Effects of DPHC and COX inhibitors on PGE\textsubscript{2} production

The results thus far indicated that DPHC increased the expression of prostaglandin synthases, i.e., COX-1, and COX-2. We therefore examined PGE\textsubscript{2} levels in cells co-treated with DPHC and COX inhibitors to confirm the role of these enzymes in the enhanced PGE\textsubscript{2} expression in HaCaT keratinocytes. Indomethacin is a strong non-selective COX inhibitor, while rofecoxib selectively inhibits COX-2. Both inhibitors caused a decrease in DPHC-induced PGE\textsubscript{2} levels. These results confirmed the roles of COX-1 and COX-2 in the observed increase in PGE\textsubscript{2} production (Fig. 4).

DISCUSSION

In the COX pathway, several PGs are produced. These autocrine or paracrine mediators signal changes within the immediate cell environment by binding to prostaglandin receptors linked to a cAMP signal transduction pathway (Dubois et al., 1998; Malde et al., 2000). The numerous biological activities of PGE\textsubscript{2} include the maintenance of local homeostasis, the relaxation of vascular smooth muscle cells, and the enhancement of blood flow. Clinically, PGE\textsubscript{2} and PGF\textsubscript{2} are used in obstetrics to induce labor.

In our experimental system consisting of HaCaT keratinocytes, both MXD and DPHC significantly increased PGE\textsubscript{2} levels. MXD is well-known to slow down the hair loss and promote the hair growth as well as an anti-hypertensive agent. Its mechanism of activity involves the opening of ATP-sensitive K\textsuperscript{-}channels (Hamaoka et al., 1997; Shorter et al., 2008), but it has also been show to up-regulate vascular endothelial growth factor (VEGF) (Lachgar et al., 1998) in dermal papilla cells. In previous experiments, several compounds isolated from Jeju seaweeds individually showed weak effects in the same assay system used in the present study (data not shown). In 2009, Kim et al. reported that an ethanolic extract of I. okamurae had anti-inflammatory effects by inhibiting NF-\kappaB. Based on the introduction and these findings, we investigated the effect of DPHC on PGE\textsubscript{2} production in macrophages (RAW264.7 cells) stimulated with LPS (1 \mu g/ml). In that study, DPHC failed to inhibit PGE\textsubscript{2} production (data not shown), suggesting that the major anti-inflammatory agent in the ethanol extract prepared by Kim et al. (2009) was not DPHC.

The expression of both COX-1 and COX-2 is induced by the PG precursor arachidonic acid as well as its PG end-products. COX-1 is constitutively expressed in most cells and tissues, and synthesizes those PGs that regulate cellular physiological activities. By contrast, COX-2 is inducible and it is functionally involved in more specialized reactions, such as inflammation, fever, pain, and cancer (Dubois et al., 1998; Warner and Mitchell, 2004). The differences in these two enzymes are reflected in their differential response to DPHC. DPHC-induced COX-1 expression occurred at a relatively early time point (3 hr), whereas COX-2 induction occurred at a later time point (12 hr) and required a higher concentration of the phlorotannin.

The intracellular accumulation of PGE\textsubscript{2} is regulated not only by its synthesis but also by its degradation. One of the key enzymes involved in PG catabolism is nicotinamide adenine dinucleotide (NAD\textsuperscript{+}-dependent 15-PGDH. This enzyme, which is widely distributed in various mammalian tissues (Tai et al., 2006), catalyzes the oxidation of the 15-hydroxy group of PGE\textsubscript{2} to yield 15-keto PGE\textsubscript{2}, and plays an essential role in the biological inactivation of PGE\textsubscript{2}. However, there was no change in 15-PGDH mRNA expression in cells exposed for 24 hr to 50 \mu g DPHC/ml (Fig. 3C). Based on these results, COX-1 appears to be the major enzyme leading to PGE\textsubscript{2} production.

We lastly confirmed the influence of each COXs induced by DPHC on the PGE\textsubscript{2} production. In 1971, aspirin, salicylate, and indomethacin were shown to inhibit PG synthesis via the inhibition of COX (PGHS) (Vane, 1971). In 1991, a second COX isoform was discovered, COX-2, and its induction in reactions related to inflammation, fever, pain, and stress adaptation was determined. The discovery of COX-2 was followed by the development of COX-2 selective inhibitors (meloxicam, celecoxib, and rofecoxib, etc.). Nonetheless, the effects of these synthases are complex and their respective activities have been difficult to distinguish. Consequently, we examined the PGE\textsubscript{2} level in cultured human keratinocytes treated with DPHC and either the non-selective COX inhibitor indomethacin or the selective COX-2 inhibitor rofecoxib. Both inhibitors significantly decreased the DPHC-mediated increase in PGE\textsubscript{2} levels, suggesting the involvement of COX-1 as well as COX-2; however, due to the different potencies of indomethacin vs. rofecoxib, it remains unclear which is the major enzyme in PGE\textsubscript{2} production.

In conclusion, DPHC, a phlorotannin isolated from I. okamurae, increased PGE\textsubscript{2} production by stimulating COX expression in HaCaT keratinocytes and might be beneficial compound for the treatment of hair regrowth.

ACKNOWLEDGMENTS

This research was part of the project titled “Development of product and material hair-growth from Jeju marine resources” funded by the Ministry of Land, Transport and Maritime Affairs, Korea.

REFERENCES

Ahn, M., Moon, C., Yang, W., Ko, E. J., Hyun, J. W., Joo, H. G., Jee,
Y., Lee, N. H., Park, J. W., Ko, R. K., Kim, G. O. and Shin, T. (2011) Diphloethohydroxycarmalol isolated from the brown algae *Ishige okamurae*, protects against radiation-induced cell damage in mice. *Food Chem. Toxicol.* *49*, 864-870.

Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van De Putte, L. B. and Lipsky, P. E. (1998) Cyclooxygenase in biology and disease. *FASEB J.* *12*, 1063-1073.

Flower, R. J. (2006) Prostaglandins, bioassay and inflammation. *Br. J. Pharmacol.* *147 Suppl 1*, S182-192.

Giuliano, F. and Warner, T. D. (2002) Origins of prostaglandin E2: involvements of cyclooxygenase (COX)-1 and COX-2 in human and rat systems. *J. Pharmacol. Exp. Ther.* *303*, 1001-1006.

Hamaoka, H., Minakuchi, K., Miyoshi, H., Arase, S., Chen, C. H. and Nakaya, Y. (1997) Effect of K+ channel openers on K+ channel in cultured human dermal papilla cells. *J. Med. Invest.* *44*, 73-77.

Harris, S. G., Padilla, J., Kounnas, L., Ray, D. and Phipps, R. P. (2002) Prostaglandins as modulators of immunity. *Trends Immunol.* *23*, 144-150.

Heo, S. J., Hwang, J. Y., Choi, J. I., Han, J. S., Kim, H. J. and Jeon, Y. J. (2009) Diphloethohydroxycarmalol isolated from *Ishige okamurae*, a brown algae, a potent alpha-glucosidase and alpha-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. *Eur. J. Pharmacol.* *615*, 252-256.

Heo, S. J., Kim, J. P., Jung, W. K., Lee, N. H., Kang, H. S., Jun, E. M., Park, S. H., Kang, S. M., Lee, Y. J., Park, P. J. and Jeon, Y. J. (2008) Identification of chemical structure and free radical scavenging activity of diphloethohydroxycarmalol isolated from a brown alga, *Ishige okamurae*. *J. Microbiol. Biotechnol.* *18*, 676-681.

Heo, S. J., Ko, S. C., Kang, S. M., Cha, S. H., Lee, S. H., Kang, D. H., Jung, W. K., Affan, A., Oh, C. and Jeon, Y. J. (2010) Inhibitory effect of diphloethohydroxycarmalol on melanogenesis and its protective effect against UV-B radiation-induced cell damage. *Food Chem. Toxicol.* *48*, 1355-1361.

Hoggaat, J., Singh, P., Sampath, J. and Pelus, L. M. (2009) Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* *113*, 5444-5455.

Johnstone, M. A. and Albert, D. M. (2002) Prostaglandin-induced hair growth. *Surv. Ophthalmol.* *47* Supple 1, S185-202.

Kim, M. M., Rajapakse, N. and Kim, S. K. (2009) Anti-inflammatory effect of *Ishige okamurae* ethanolic extract via inhibition of NF-kappaB transcription factor in RAW 264.7 cells. *Phytother. Res.* *23*, 628-634.

Lachgar, S., Charveron, M., Gall, Y. and Bonafe, J. L. (1998) Minoxidil upregulates the expression of vascular endothelial growth factor in human hair dermal papilla cells. *Br. J. Dermatol.* *138*, 407-411.

Langenbach, R., Loftin, C., Lee, C. and Tiano, H. (1999) Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. *Biochem. Pharmacol.* *58*, 1237-1246.

Maldve, R. E., Kim, Y., Muga, S. J. and Fischer, S. M. (2000) Prostaglandin E(2) regulation of cyclooxygenase expression in keratinocytes is mediated via cyclic nucleotide-linked prostaglandin receptors. *J. Lipid Res.* *41*, 873-881.

Michelet, J. F., Commo, S., Billoni, N., Mahé, Y. F. and Bernard, B. A. (1997) Activation of cytoprotective prostaglandin synthase-1 by minoxidil as a possible explanation for its hair growth-stimulating effect. *J. Invest. Dermatol.* *108*, 205-209.

Ricciotti, E. and FitzGerald, G. A. (2011) Prostaglandins and inflammation. *Arterioscler. Thromb. Vasc. Biol.* *31*, 986-1000.

Sasaki, S., Hozumi, Y. and Kondo, S. (2005) Influence of prostaglandin F2alpha and its analogues on hair regrowth and follicular melanogenesis in a murine model. *Exp. Dermatol.* *14*, 323-328.

Shorter, K., Farjo, N. P., Pickles, S. M. and Randall, V. A. (2008) Human hair follicles contain two forms of ATP-sensitive potassium channels, only one of which is sensitive to minoxidil. *FASEB J.* *22*, 1725-1736.

Tai, H. H., Chi, X. and Tong, M. (2011) Regulation of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) by non-steroidal anti-inflammatory drugs (NSAIDs). *Prostaglandins Other Lipid Mediat.* *96*, 37-40.

Tai, H. H., Cho, H., Tong, M. and Ding, Y. (2006) NAD+-linked 15-hydroxyprostaglandin dehydrogenase: structure and biological functions. *Curr. Pharm. Des.* *12*, 955-962.

Tanabe, T. and Tohnai, N. (2002) Cyclooxygenase isozymes and their gene structures and expression. *Prostaglandins Other Lipid Mediat.* *68-69*, 95-114.

Vane, J. R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* *231*, 232-235.

Warner, T. D. and Mitchell, J. A. (2004) Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB J.* *18*, 790-804.