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Triterpenoid from *Tiarella polyphylla*, Regulation of Type 1 Procollagen and MMP-1 in Ultraviolet Irradiation of Cultured Old Age Human Dermal Fibroblasts

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Although many studies have been performed to elucidate the molecular consequences of ultraviolet irradiation, little is known about the effect of natural products. Ultraviolet irradiation is widely considered to be an environmental stress. Here we investigated the effect of 3,23-dihydroxy-20(29)-lupen-27-oic acid on the regulation of MMP-1 and type 1 procollagen in Ultraviolet irradiation of cultured old age human dermal fibroblasts. 3, 23-dihydroxy-20(29)-lupen-27-oic acid was isolated from *Tiarella polyphylla* D. Don (Saxifragaceae). Among them, 3, 23-dihydroxy-20(29)-lupen-27-oic acid induced the regulation of Type 1- procollagen and reduced the regulation of MMP-1 at the protein levels in a dose-dependent manner by ultraviolet irradiation. Taken together, our results suggest that 3, 23-dihydroxy-20(29)-lupen-27-oic acid plays an important role in the induction of Type 1-procollagen and reduction of MMP-1 by ultraviolet irradiation in old age human dermal fibroblasts.

Key words: *Tiarella polyphylla*, 3, 23-Dihydroxy-20(29)-lupen-27-oic acid, MMP-1, Type 1-procollagen, Ultraviolet irradiation, Old age human dermal fibroblasts

INTRODUCTION

The matrix metalloproteinases (MMPs) are a family of >20 zinc-dependent endoproteinases that are capable of degrading almost all of the components of the extracellular matrix (Chambers *et al.*, 1997). MMPs can be divided into four categories based on substrate preference: collagenases, gelatinases, stromelysins, and membrane-associated matrix metalloproteinases (Bernhard *et al.*, 1994).

MMPs are necessary for tissue remodeling and the healing cascade under normal physiological condition. The aging process of skin can be divided into intrinsic aging and photoaging. Clinically, naturally aged skin is smooth, pale, and finely wrinkled. In contrast, photoaged skin is coarsely wrinkled (Gilchrest, 1989). Alterations in collagen, the major structural component of skin, have been suggested as a cause of the changes, such as skin wrinkling and loss of elasticity, observed in naturally aged and photoaged skin (Fisher *et al.*, 1996; Varani *et al.*, 2000). With increasing age, collagen synthesis becomes lower and MMP-1 levels become higher in sun-protected human skin *in vivo* (Varani *et al.*, 2000). UV irradiation induces the synthesis of matrix metalloportienases (MMP) skin fibroblasts *in vitro* and MMP-mediated collagen destruction accounts, in large part, for the connective tissue damage that occurs in photoaging (Fisher *et al.*, 1996).

Tiarella polyphylla D. Don (Saxifragaceae) is the only species of this genus to be found in Korea, and occurs naturally in Ullung Island (Lee, 1996). The whole plants of *T. polyphylla* has been traditionally used for the treatment of asthma and skin eruptions, Previous pharmacological study on *T. polyphylla* reported anticomplement activity (Park *et al.*, 1999) and triterpene compounds (Park *et al.*, 2002).

In this paper, we report a compound from the whole plants of *T. polyphylla* which induced the regulation of

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Type 1-procollagen and reduced the regulation of MMP-1 on the UV-induced damage of cultured old age human dermal fibroblasts.

MATERIALS AND METHODS

General experimentals

The melting points were determined using a Fisher Scientific melting point apparatus and were uncorrected. The optical rotations were measured using an Autopol-IV polarimeter. The NMR spectra were recorded on a Varian Unity Inova 500 spectrometer. The chemical shifts are expressed in parts per million (ppm) relative to TMS as the internal standard, and the coupling constants (*J*) are given in hertz. EIMS spectra were taken from Hewlett-Packard 5889A. prep HPLC was JAI LC-908 model with refractive index detector, UV detector

and Alltech Econosil Silica 10 mm column (250 mm×22 mm). TLC and the preparative TLC were carried out on precoated Silica gel F_{254} (Merck, art. 5715) and RP-18 F_{254}s (Merck, art. 15389) plates. Column chromatography was performed on Silica gel 60 (Merck, 40-63 and 63-200 mm) and Sephadex LH-20 (Sigma, 25-100 mm).

Plant material

The whole plants of *Tiarella polyphylla* were collected in April 2004 at Ullung island, Korea. and The botanical identification was made by one of the authors, Dr. Joongku Lee. A voucher specimen of this raw material has been deposited at the herbarium of the Seoul National University (SNU-2004-04-19-1).

Extraction and isolation

Dried and chopped whole plants (213 g) were extracted with MeOH (500 mL×5). The extract was concentrated *in vacuo* to yield a dark green residue (28.5 g), which was suspended in water and successively partitioned with EtOAc extracts (6.3 g), was subjected to column chromatography on silica gel eluting with hexane followed by hexane-EtOAc mixtures of increasing polarity and finally with $CHCl_3$-MeOH (1:1) mixture. Six fractions were obtained from the EtOAc layer. Fraction IV was purified with silica gel ($CHCl_3$-MeOH = 9.5:0.5) and Sephadex LH-20 column chromatography using methylene chloride/methanol (1:5). Preparative HPLC (methylene chloride/methanol/water 70:10:1) to afford compound (6 mg).

Tiarellic acid

White Needles (MeOH), mp 256-257°C (decomposed); $[\alpha]_D^{23}$ + 86° (pyridine; c 0.13); EI-MS (70 eV) m/z (rel. int.): 472 [M^+] (61), 454 [M^+-H_2O] (32), 436 [M^+-$2H_2O$] (60), 173 (100), 81 (23); ^{1}H-NMR and ^{13}C-NMR (pyridine-d_5): see Table I.

Table I. NMR data of Tiarellic acid (δ in ppm)

Position	δ^{13}C	δ^1H (*J*, Hz)	HMBC (C-H)
1	39.19	α 1.04 dd (2.9, 12.5, 13.0) β 1.69, m	5, 25
2	27.82	α 1.81 m, β 1.90 m	1, 3
3	73.56	4.02, dd (4.6, 11.6)	1, 2, 5, 23, 24
4	42.87	--	3, 5, 23, 24
5	49.23	α 1.50, dd (1.5, 12.0)	1, 6, 9, 23, 24, 25
6	18.68	α 1.65, dddd (1.5, 1.5, 3.0, 13.0) β 1.49, dddd (1.7, 12.0, 12.5, 13.0)	5, 7
7	38.18	α 2.06, ddd (3.0, 12.5, 13.0) β 1.87, ddd (1.5, 1.7, 13.0)	26
8	40.83	--	7, 9, 26
9	51.57	α 2.02, dd (1.7, 12.7)	5, 11, 25, 26
10	37.70	--	1, 5, 9, 25
11	21.24	α 1.64, m, β 1.32, dddd (4.4, 12.7, 13.0, 13.1)	9, 12
12	26.73	α 2.58, m, β 1.86, m	9, 11, 13
13	39.52	β 1.88, m	12, 15, 18
14	60.35	--	7, 9, 13, 15, 16, 18, 26
15	25.82	α 2.28, ddd (3.0, 3.0, 13.1), β 1.67, m	16, 26
16	38.29	α 1.78, m, β 1.70, m	15, 28
17	42.95	--	16, 18, 22, 28
18	51.21	α 1.81, m	12, 13, 19, 21, 22
19	48.14	β 2.60, m	18, 21, 22, 29, 30
20	150.99	--	18, 19, 21, 29, 30
21	30.12	α 1.36, m, β 1.97, m	19, 22
22	40.39	α 1.13, ddd (10.5, 10.5, 10.5) β 1.38, dd (10.2, 10.5)	21
23	68.20	3.57, d (10.4), 4.07, d (10.4)	3, 5, 24
24	12.98	1.03, s	3, 5, 23
25	17.41	1.01, s	5, 9
26	17.52	1.19, s	9
27	178.28	--	13, 15
28	18.84	1.0, s	18, 22
29	110.17	4.76, s 4.93, s	19, 30
30	19.41	1.84, s	19, 29

Old age human dermal fibroblasts cell culture

Primary cultures of old age human dermal fibroblasts were established from 72 age human forearm skin in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum, 2 mM glutamine, and penicillin (100 U/mL), streptomycin (100 μg/mL) in a 37°C humidified incubator containing 5% CO_2. The fibroblasts were cultured until 90% confluency and then, subcultivated. Cells cultured after 5 passages were used for the experiments.

UV irradiation

The UV light source was a F75/85W/UV21 fluorescent

sun lamps, having an emission spectrum between 285-350 nm (peak at 310-315 nm) as previously described (Seo *et al.*, 2001). A Kodacel filter (TA401/407; Kodak, Rochester, NY) was mounted 2 cm in front of the UV tubes to remove wavelengths <290 nm (UV-C). The fibroblasts were grown in 10 cm culture dishes (Falcon, Lincoln Park, NJ) until subconfluent. Subsequently, the cells were cultured in serum-free medium for 24 h, and the medium was replaced by 2 mL of phosphate-buffered saline. Then the cells were exposed to UV (0-100 mJ/cm^2) light. After irradiation, the cells were washed with phosphate-buffered saline, and cultured in the media with or without compounds for the indicated time.

Cell proliferation assay

Cell proliferation was determined by the MTT assay (Mosmann, 1983), which is based on reduction of soluble yellow MTT tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to a blue insoluble MTT formazan product by mitochondrial succinic dehydrogenase. After compound or UV treatment, the cells were cultured for indicated days; 20 μL of MTT (5 mg/mL) was added to each well, and the cells were incubated for 4 h at 37°C. The supernatant was removed, and 200 μL of dimethylsulfoxide was added to each well to dissolve formazan products. The absorbance was determined spectrophotometrically at 570 nm with an ELISA reader. The results were expressed as a percentage of control in six cultures.

Western blot and statistical analysis

Supernatant extract were centrifuged at 12,000×g for 10 min, and used for western blot analysis. A monoclonal anti-type I procollagen aminoterminal extension peptide (SP1.D8) antibody (Developmental Studies Hybridoma Bank, Iowa City, IA) and monoclonal anti-MMP-1 antibody (Oncogen, Co., Boston, MA) were used as primary antibodies. Statistical significance was determined using the Student *t*-tests. Results are presented by means ± SEM. All p values quoted are two-tailed and were accepted as significant when p was ≤ 0.05.

RESULTS AND DISCUSSION

Activity-guided column chromatographies of a EtOAC soluble fraction from the whole plants of *T. polyphylla* led to the isolation of compound (Fig. 1). Tiarellic acid was obtained as white crystal. The EI-MS spectrum showed an [M$^+$] ion at *m/z* 472. The ^{13}C-NMR spectrum and DEPT spectra showed 30 carbon signals which were composed of five methyl, twelve methylene, six methine and seven quaternary carbon signals (Table I). All of the methyls appeared as singlet in ^{1}H-NMR spectrum, and four methyl signals were at upper field (δ 1.0-1.2) but one methyl

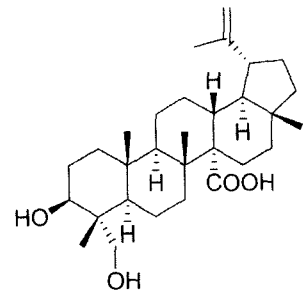

Fig. 1. Chemical structures of tiarellic acid

signal at downfield (δ 1.84). These results indicated the methyl at δ 1.84 neighbors a double-bonded carbon, and this fact was confirmed by HMBC. This methyl signal had cross peaks with the signals at 150.99 (C-20) and 110.17 (C-29, CH_2) in HMBC, which suggested compound contained a terminal olefin group. The signals at 73.56 (C-3) coupled with 4.02 (H-3) and at 68.20 (C-23) coupled with 4.07 and 3.57 (H-23) in HMQC showed this compound possessed one primary and one secondary hydroxyl group. These results showed a characteristic pattern this compound is an lupene-type triterpene. The carboxyl carbon signal of this compound showed cross peaks with H-13 and H-15 in HMBC. The methyl signal of C-28 (δ 18.84) showed cross peaks with H-18 and H-22 in HMBC. These results indicate the carboxyl group must be at C-27, respectively. Based on the NMR spectral evidences, and compound was characterized by comparing their ^{1}H- and ^{13}C-NMR data with those reported in the literatures (Park *et al.*, 2000). Compound was determined to be 3,23-dihydroxy-20(29)-lupen-27-oic acid (Tiarellic acid). Tiarellic acid did not showed cytotoxicity against old age human

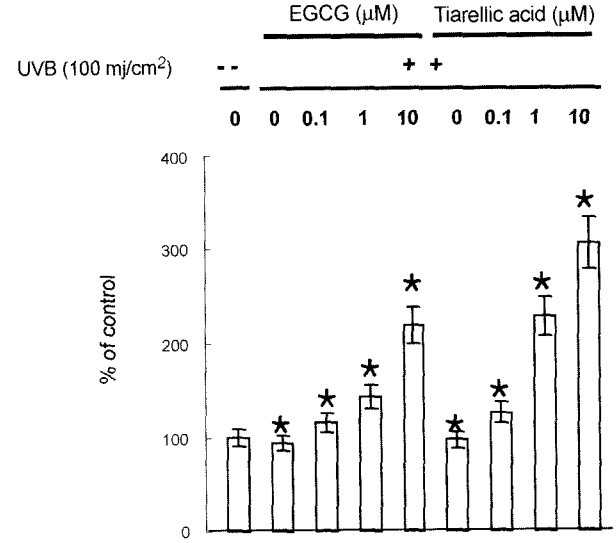

Fig. 2. Cell proliferation of old age human dermal fibroblasts in tiarellic acid treatment. a) Percent of control, significantly different from control: * P<0.05, n=5

dermal fibroblast in test dose (0.1~10 μM, $p<0.001$) as compared to control (Fig. 2). We studied the effects of tiarellic acid on the regulation of type 1 procollagen and matrix metalloproteinase-1 in ultraviolet irradiation of cultured old age human dermal fibroblasts. Fibroblasts were treated with 0.1, 1, 10 μM for 72 h and then, the regulation levels of type I procollagen and MMP-1 were determined in the culture media by western blot analysis. We demonstrated that tiarellic acid decreased the regulation of MMP-1 and increased the regulation of Type 1-procollagen at the protein levels in a dose-dependent manner by ultraviolet irradiation of cultured old age human dermal fibroblasts. To investigate the dose-dependent effects of tiarellic acid, old age human dermal fibroblasts were treated with various doses of tiarellic acid for 72 h. The type I procollagen protein regulation increased by 129.1±11% ($p<0.05$, n=5) at 0.1 μM, 183.2 ± 9% ($p<0.05$, n=5) at 1 μM, and 231.2 ± 11% ($p<0.05$, n=5) at 10 μM and decreased MMP-1 protein regulation significantly in a dose-dependent manner; by an average of 73.1 ± 10% ($p<0.05$, n=5) at 0.1 μM, 62.7 ± 10% ($p<0.05$, n=5) at 1 μM, and 60.2 ± 10% ($p<0.05$, n=5) at 10 μM, compared with UV-treated control cells (Fig. 3, 4). Tarellic acid showed similar activity to positive control, EGCG in the protein levels. In conclusion, tiarellic acid may be used for the treatment and recovery of skin damage by UV irradiation in old age human skin.

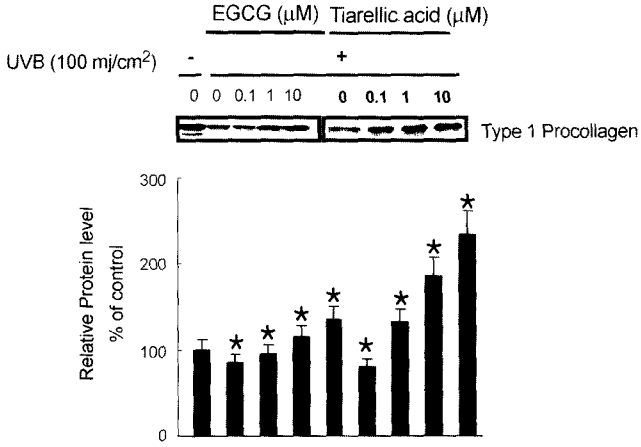

Fig. 3. The effect of tiarellic acid on the product of Type I procollagen by ultraviolet irradiation of cultured old age human dermal fibroblasts. a) Tiarellic acid induced the regulation of Type I procollagen and b) Percent of control, significantly different from control: * $P<0.05$, n=5.

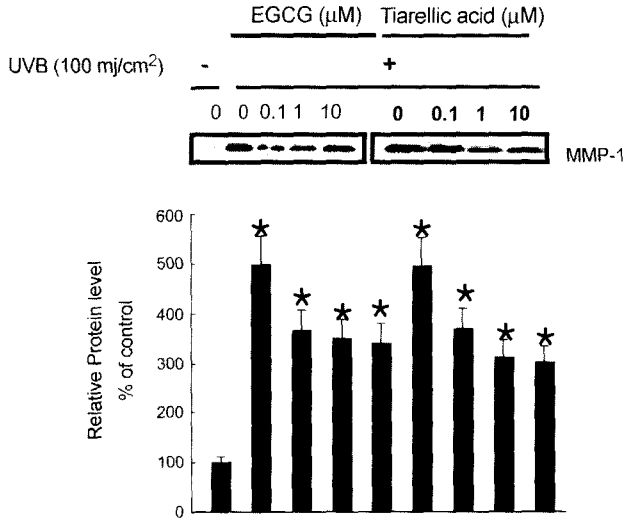

Fig. 4. The effect of tiarellic acid on the product of MMP-1 by ultraviolet irradiation of cultured old age human dermal fibroblasts. a) Tiarellic acid reduced the regulation of MMP-1 and b) Percent of control, significantly different from control: * $P<0.05$, n=5.

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