Mechanisms of carvacrol-induced expression of type I collagen gene

Jongsung Lee a,b, Eunsun Jung a, Hyunkyung Yu c, Yongwoo Kim a, Jaehyoun Ha d, Yeong Shik Kim b,*, Deokhoon Park a, **

a Biospectrum Life Science Institute, 101-701 SK VENTIUM, 522 Dangjung Dong, Gunpo City, 435-833 Gyunggi Do, Republic of Korea
b Natural Products Research Institute, College of Pharmacy, Seoul National University, Jongro Gu, Seoul, Republic of Korea
c Mogam Biotechnology Research Institute, Bojing Dong, Yongin City, 449-913 Gyunggi Do, Republic of Korea
d IEC Korea, Yeongtong Dong, Suwon City, 443-813 Gyunggi Do, Republic of Korea

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Summary

Background: Skin aging is accompanied by wrinkle formation and appears to be principally related to decreases in the levels of type I collagen, the primary component of the dermal layer of skin.

Objective: To investigate the effect of carvacrol on collagen gene expression and its mechanisms of action.

Methods: To elucidate the effect of carvacrol on collagen expression and its mechanism, several experiments were performed in human dermal fibroblasts. Collagen production, small interference RNA, Ca2+ mobilization, COL1A2/AP-1 luciferase reporter assays and Western blots for proteins that are involved in collagen gene expression were used in this study.

Results: Carvacrol activated both the human COL1A2 promoter activity and the synthesis of human type I procollagen. Additionally, we attempted to characterize the mechanism of action of carvacrol in type I procollagen synthesis. In a human COL1A2 promoter luciferase assay, the small interference RNA for SP-1 did not reduce the carvacrol-induced promoter activation. Also, Smad 2 phosphorylation was not induced by carvacrol. However, in the AP-1 (activator protein-1) luciferase reporter...
1. Introduction

Collagens are the major structural components of the skin and represent a large family of extracellular proteins that impart specific physical properties to tissues. These proteins also have important functions during morphogenesis and growth [1,2]. Type I collagen, the most abundantly expressed member of the collagens, consists of two \( \alpha_1(I) \) chains and one \( \alpha_2(I) \) chain that are produced by two fairly large genes that reside on different chromosomes in both the human and the mouse genome. Type I collagen also plays a significant role in maintaining homeostasis [3–5].

**Fig. 1** Activation of type I procollagen by carvacrol in human dermal fibroblasts. (A) Structure of carvacrol [2-methyl-5-(1-methylethyl) phenol]. (B) Effects of carvacrol on COL1A2 promoter. Stable transfectants were incubated with carvacrol for 24 h and luciferase activity was then assayed. * \( p < 0.05 \) vs. controls. Cv, carvacrol. (C) Effects of carvacrol on type I procollagen synthesis as determined using a sandwich immunoassay kit (TAKARA BIO, INC., Japan). * \( p < 0.05 \) vs. controls. (D and E) Effect of carvacrol on mRNA (D) and protein (E) levels of type I collagen. Human dermal fibroblasts were treated with or without TGF-\( \beta \) and/or carvacrol at the indicated concentrations for 24 h at 37°C. Real-time PCR (D) and Western blot analysis (E) of whole cell lysates was performed as described in Section 2. * \( p < 0.05 \), vs. untreated control.
In human skin, several signaling pathways regulate type I collagen gene expression. Thus far, three major signaling pathways have been found to induce type I collagen production; the first is the Smad-mediated pathway [6], the second is the SP-1-mediated pathway [7], and the third is the activator protein-1 (AP-1)-mediated pathway [8]. The Smads have been identified as intracellular mediators of the signal transduction pathways of the TGF-β superfamily members, functioning downstream of the serine/threonine kinase receptors of the TGF-β family to transduce signals to the nucleus [9—11]. Smad3 promotes the activation of the α2(1) collagen gene (COL1A2) [12].

SP-1 binding is required for the expression of type I collagen mRNA. SP-1 and Smad proteins form complexes, and their synergy plays an important role in mediating type I collagen expression. SP-1- and Smad-binding elements are present in the COL1A2 promoter region. Removal of SP-1- and Smad-binding elements inhibits the stimulation of COL1A2 promoter activity.

It has been reported that AP-1 modulates the expression of the type I collagen gene in skin fibroblasts [13,14], and up-regulation of AP-1-binding activity also correlates with increased expression of the type I collagen gene [8,15]. In addition, the AP-1-binding element is present in the COL1A2 promoter region. Therefore, AP-1 may play an important role in the regulation of collagen synthesis.

Carvacrol [2-methyl-5-(1-methylethyl) phenol] is a monoterpenic phenol with substantial antibacterial, antifungal, and insecticidal effects (Fig. 1A) and has been safely used as a cosmetic ingredient. Recent studies revealed that this compound suppresses the growth of mouse B16 melanomas in vitro [16] and inhibits DNA synthesis in mouse myoblasts bearing a human N-ras oncogene, suggesting that carvacrol may act as an anti-cancer agent [17]. Due to the properties of carvacrol, it has a broad range of applications in products ranging from food preservatives to cosmetics. Although many reports on the anti-carcinogenic and anti-microbial effects of carvacrol have been published, its effect on skin aging remains unknown.

Aging of the skin is fundamentally related to reductions in the levels of type I collagen, the principal component of the dermal layer of the skin. Type I collagen is the main structural component of the extracellular matrix (ECM), which performs a pivotal function in maintaining the structure of the dermis. An efficient agent is needed for effective management of skin aging; this agent should have the fewest possible side effects and the greatest wrinkle-reducing effects. We obtained carvacrol in the course of screening for collagen production-promoting agents. This study was designed to investigate the possible collagen production-promoting activities of carvacrol and its mechanism of action in human dermal fibroblasts.

2. Materials and methods

2.1. Materials

Dulbecco’s modified Eagle’s medium (DMEM), anti-collagen type I antibody (C-18), and anti-Smad2 (S-20) antibody were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-phospho-p38 mitogen-activated protein kinases (MAPK) antibody (Thr180/Tyr182) (28B10), anti-phospho-JNK antibody, anti-phospho-ERK1/2 (p42/44 MAPK) antibody (Thr202/Tyr204) (E10), anti-phospho-Smad2 (Ser465/467) antibody and anti-phospho-phospholipase Cγ1 (PLCγ1) antibody (Tyr783) were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Phenylarsine oxide (PAO), PD98059, SP600125, SB203580 and human TGF-β were purchased from Sigma Chemical Co. (St. Louis, MO). Protease inhibitor cocktail was purchased from Roche (Indianapolis, IN). Chemiluminescence kits were purchased from Amersham Pharmacia Biotech (Buckinghamshire, England). AP-Luc reporter plasmid was purchased from Stratagene (West Cedar Creek, TX).

2.2. Cell culture

Human dermal fibroblasts were obtained from AmorePacific and were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The cells were grown at 37 °C in a 95% air/5% CO2 environment. Transfected human dermal fibroblasts were incubated with 800 μg/ml of neomycin (100 mg/ml stock) for the selection and maintenance of stable transfectants.

2.3. Small interference RNA (siRNA)

siRNA cocktails targeting SP-1 were purchased from B-Bridge International (Tokyo, Japan). The sequences for this siRNA duplex were as follows: gcagaaagagagcaagTT, cggagagcucagcaggaTT, and ccagaagagugagauaTT. A negative control siRNA cocktail was also purchased from B-Bridge International. Human dermal fibroblasts were transfected using Superfect transfection reagent (Invtrogen, Carlsbad, CA) with a final concentration of
50 nM siRNA. Experiments were repeated four times. Data are expressed as means ± S.D.

2.4. Cell transfection and COL1A2 luciferase assay

Human dermal fibroblasts were co-transfected with the recombinant expression vector pLuc-COL1A2 and pCI-neo plasmid using Superfect transfection reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s suggested protocol. After 24 h, stable transfectants were selected with 800 µg/ml G418 and isolated. The transfectants were maintained in DMEM containing 10% fetal bovine serum and 300 µg/ml G418 [18].

One milliliter (1 × 10⁵ cells) of each stable transfectant was plated in 12-well plates. After 24 h, carvacrol or TGF-β was added for 24 h. Cells were then lysed and 120 µg of lysate was used to measure reporter gene expression. Luciferase activity was assayed with the Luciferase Reagent Assay System (Promega, Madison, WI). Luminescence was measured at 450 nm using a 96-well plate luminometer (Berthold, Germany). Experiments were repeated four times. Data are expressed as means ± S.D.

2.5. AP-1 luciferase assay

Human dermal fibroblasts were transiently co-transfected with 2 µg of the firefly luciferase reporter gene under the control of AP-1 responsive elements (AP-1 Luc reporter) and with 0.2 µg of Renilla luciferase expression vector driven by the thymidine kinase promoter using Superfect™ reagent (Invitrogen). After 24 h, the cells were stimulated with the indicated concentrations of carvacrol for an additional 24 h. Luciferase activity is expressed as the ratio of AP-1-dependent firefly luciferase activity divided by the control thymidine kinase Renilla luciferase activity (relative luciferase units). Data are expressed as means ± S.D. The experiments were repeated eight times.

2.6. Type I collagen synthesis assay

Human dermal fibroblasts (1 × 10⁵ cells) were seeded into 24-well plates and the medium was decanted 24 h later. The cells were then washed twice with phosphate-buffered saline (PBS) and serum-free medium was added. After 24 h, carvacrol or TGF-β was added for 24 h. The culture medium was collected after stimulation and used to assess collagen synthesis. The collagen content was determined by an enzyme-linked immunosorbent assay (ELISA) using anti-type I collagen antibody (Takara Bio Inc., Japan). The results were verified by four repetitions of the experiments, each in triplicate. Data are expressed as means ± S.D.

2.7. Immunoprecipitation of smad protein

Smad immunoprecipitation and blotting were conducted as previously described [19]. Briefly, fibroblasts (1 × 10⁶ cells) were seeded in six-well plates and cultured for 24 h. The medium was then replaced with HEPES buffer (1 M) for 2 h. The cells were subsequently treated with TGF-β or carvacrol for 30 min. The fibroblasts were rinsed twice with ice-cold PBS and were harvested in RIPA buffer (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 1 µg/ml pepstatin) and phosphatase inhibitors (1 mM sodium orthovanadate, 50 mM NaF, 40 mM β-glycerophosphate). The lysates were centrifuged at 14,000 × g for 30 min. 100 µg of lysate proteins were immunoprecipitated overnight at 4 °C with 0.2 µG of anti-Smad 2 antibodies (Smad2 (S-20) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), followed by precipitation using 10 µl of protein G-agarose at 4 °C for 90 min. After four washes with complete RIPA buffer, the immunoprecipitates were eluted by boiling for 5 min in 60 µl of SDS sample buffer (100 mM Tris/HCl, pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 200 mM dithiothreitol). The experiments were repeated three times. Data are expressed as means ± S.D.

2.8. AP-1 binding assay

Human dermal fibroblasts were treated with PMA and/or carvacrol at the indicated concentrations for 40 min. For nuclear protein isolation, the NucBuster Protein Extraction kit (Novagen) was used according to the manufacturer’s instructions, and the nuclear pellets were resuspended in 50 µl of complete lysis buffer provided in the Active Motif kit. After 30-min incubation on ice, samples were centrifuged and protein concentrations were measured with the Bio-Rad Protein Assay kit. The AP-1 consensus nucleotide binding activity in the nuclear extracts (10 µg) was measured using the TransAM AP-1 family (Active Motif, Carlsbad, CA) colorimetric transcription factor measurement system as recommended. Briefly, nuclear extract was added to the immobilized oligonucleotides, followed by addition of primary transcription factor antibody, secondary horse radish peroxidase (HRP)-conjugated antibody, and HRP substrate. Colorimetric values measured at 450 nm were plotted as bar graphs. As a control for specificity, excess wild-type consensus and mutant
AP-1 oligonucleotides were used as competitors for AP-1 binding. All experiments were performed three times. Data are expressed as means ± S.D.

2.9. Immunoblotting

Cells (1 × 10^6 cells) were plated in six-well plastic tissue culture dishes and grown to subconfluence. After 24 h of quiescence, cells were treated with or without PMA/TGF-β and/or carvacrol at the indicated concentrations for 15 min or 24 h at 37 °C. Cells were then washed twice with cold PBS and lysed in 150 μl of sample buffer (100 mM Tris—HCl, pH 6.8, 10% glycerol, 4% sodium dodecyl sulfate [SDS], 1% bromophenol blue, 10% β-mercaptoethanol). The samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Immobilon-P PVDF membranes (Millipore Corporation, Bedford, MA, USA). The membranes were incubated overnight at 4 °C with anti-phospho-p38 MAPK antibody (Thr180/Tyr182) (28B10), anti-phospho-JNK antibody, anti-phospho-ERK1/2 antibody (Thr202/Tyr204) (E10), anti-phospholipase Cγ1 antibody (Tyr783), anti-collagen type I antibody (C-18) or anti-β-actin antibody. The membranes were washed three times with Tris-buffered saline containing Tween-20 (Sigma) (TBST), probed with horseradish peroxidase-conjugated secondary antibody, and developed using an ECL (enhanced chemiluminescence) Western blotting detection system (Amersham Biosciences). The experiments were repeated three times. Data are expressed as means ± S.D.

2.10. Measurement of transient intracellular Ca^{2+}

[Ca^{2+}], was measured using a calcium-sensitive fluorescent dye, Fura2-AM. Human dermal fibroblasts were washed with Krebs—HEPES buffer, which contained 120 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl_2, 1 mM NaH_2PO_4 and 10 mM HEPES (pH 7.4), and subsequently incubated with Fura2-AM at 37 °C for 1 h. The cells were then washed with Krebs—HEPES buffer and resuspended at 1 × 10^6 cells/ml in the same buffer. Three milliliters of the cell suspension was placed in a quartz cuvette in a luminescence spectrometer (PerkinElmer, LS50B) and fluorescence was monitored at 340 nm (λ_ex1), 380 nm (λ_ex2) and 510 nm (λ_em) every 5 s for 100 s.

2.11. RNA isolation and quantitative real-time RT-PCR

Cells (1 × 10^6 cells) were plated in six-well plastic tissue culture dishes and grown to subconfluence. After 24 h of quiescence, cells were treated with or without TGF-β and/or carvacrol at the indicated concentrations for 24 h at 37 °C. Total RNA was extracted from cultured human dermal fibroblasts using TRIZOL® Reagent (Invitrogen, Carlsbad, CA). The levels of mRNA expression were quantified by real-time RT-PCR using TaKaRa SYBR® EXScript™ RT-PCR Kit (TAKARA BIO INC., Japan) and 7300 real-time PCR System (Applied Biosystems, Foster City, CA). Primers for type I procollagen real-time PCR were as follows: Type I procollagen sense primer, 5'-CGGAC-GACCTGGTGAGAGA-3'; Type I procollagen antisense primer, 5'-CATTGTGTTCCCTATGCTT-3'. To compare with treatment groups, the type I procollagen level was quantified based on standard curves and normalized to levels of the housekeeping gene GAPDH as an internal control. The experiments were repeated three times. Data are expressed as means ± S.D.

2.12. Statistical evaluation

Averages ± S.E.M. were calculated; statistical analysis of the results was performed using Student’s t-test for independent samples. Values of *p < 0.05 were considered significant.

3. Results

3.1. Activation of type I procollagen by carvacrol in human dermal fibroblasts

Our understanding of the expression of the human α2(I) collagen (COL1A2) gene and of its transcriptional regulation by cytokines and growth factors has improved significantly in recent years. In order to determine whether carvacrol affects collagen production, we performed COL1A2 luciferase and type I procollagen production assays. As shown in Fig. 1B and C, carvacrol increased both COL1A2 reporter activity and the production of type I procollagen in a concentration-dependent manner. This result was further confirmed by real-time PCR and Western blotting (Fig. 1D and E). TGF-β was employed as a positive control, and in an MTT assay of human dermal fibroblasts, we found that carvacrol had no significant cytotoxic effect at any of the tested concentrations (data not shown).

3.2. Activation of JNK and ERK1/2 by carvacrol

AP-1 modulates the expression of the type I collagen gene in skin fibroblasts, and up-regulation of AP-1-binding activity is also correlated with increased
Carvacrol induces type I collagen gene expression

Fig. 2   Activation of both AP-1 and MAPK by carvacrol. (A) To determine the effect of carvacrol on the AP-1 promoter, human dermal fibroblasts were transiently co-transfected with the AP-1 Luc reporter and with Renilla luciferase expression vector, as described in Section 2. After 24 h, the cells were stimulated with the indicated concentrations of carvacrol for an additional 24 h. Luciferase activity was then measured. *p < 0.05 vs. controls. Cv: carvacrol. (B) Effects of carvacrol on AP-1 binding activity. Human dermal fibroblasts were treated with or without PMA and/or carvacrol at the indicated concentrations for 40 min at 37 °C. AP-1 DNA-binding activity in the nuclear extracts was then measured by AP-1-specific ELISA. *p < 0.05, vs. untreated control. (C) Effects of carvacrol on MAPKs. Human dermal fibroblasts were treated with or without PMA and/or carvacrol at the indicated concentrations for 15 min at 37 °C. Western blot analysis of whole cell lysates was performed as described in Section 2. *p < 0.05 vs. untreated control. (D) Effect of inhibitors on carvacrol-induced AP-1 promoter activation. Human dermal fibroblasts were transiently co-transfected with the AP-1 Luc reporter and with Renilla luciferase expression. After 24 h, cells were pretreated with the indicated inhibitors for 1 h and then stimulated with carvacrol or PMA for an additional 24 h. Luciferase activity was then measured. *p < 0.05 vs. PMA-treated controls; *p < 0.05 vs. carvacrol-treated controls. PD, PD98059; SP, SP600125; SB, SB203580.
expression of the type I collagen gene in response to TGF-β [8,13,14,15]. Therefore, we hypothesized that carvacrol-induced collagen production was dependent on the AP-1 activation pathway. In order to test this, we performed AP-1 promoter-luciferase reporter assays, AP-1 binding assays, and Western blotting for the phosphorylated forms of the MAPKs, JNK, p38 MAPK, and ERK1/2. As shown in Fig. 2A and B, carvacrol increased the AP-1 promoter and binding activities in a concentration-dependent manner. Excessive wild-type consensus oligonucleotide that served as competitor for AP-1 binding prevented binding of carvacrol-stimulated nuclear extract with immobilized AP-1 DNA on the plate, while mutant oligonucleotide did not, thus demonstrating the specificity of the assay (Fig. 2B). This finding was further confirmed by Western blotting for the phosphorylated forms of the MAPKs. We found that carvacrol induced phosphorylation of JNK and ERK1/2, but did not induce p38 MAPK phosphorylation (Fig. 2C). In addition, carvacrol-induced activation of AP-1 promoter was inhibited by PD98059 (a specific ERK1/2 inhibitor) and SP600125 (a specific JNK inhibitor), but not by SB203580 (a specific p38 inhibitor) (Fig. 2D). However, the Smad and SP-1 activation pathways may also be involved in collagen production. Therefore, we investigated the effect of carvacrol on Smad 2 and SP-1. In this study, we found that carvacrol had no effect on the phosphorylation of Smad 2 (Fig. 3A). In addition, knockdown of SP-1 expression in cultured human dermal fibroblasts using small interference RNA (siRNA) specific for SP-1 did not reduce the COL1A2 luciferase activity induced by carvacrol (Fig. 3B).

3.3. Intracellular Ca²⁺ mobilization and induction of PLCγ1 phosphorylation by carvacrol

Ca²⁺ is among the signals that induce MAPK activation. Therefore, we examined whether carvacrol can induce intracellular Ca²⁺ mobilization in human dermal fibroblasts. Real-time changes in the level of intracellular Ca²⁺ were measured (Fig. 4A). Treatment of human dermal fibroblasts with carvacrol effectively induced an elevation in the Ca²⁺ level.

PLC hydrolyzes phosphatidylinositol-4,5-bisphosphate to generate inositol-1,4,5-trisphosphate (IP₃) and 1,2-diacylglycerol (DAG), which are involved in the mobilization of intracellular Ca²⁺ and the activation of protein kinase C, respectively [20]. Therefore, we investigated the effect of carvacrol on the tyrosine phosphorylation of PLCγ1. As shown in Fig. 4B, PLCγ1 was tyrosine-phosphorylated in a concentration-dependent manner upon addition of carvacrol. Furthermore, while PAO (a PLCγ1 inhibitor), PD98059 (a specific ERK1/2 inhibitor) and SP600125 (a specific JNK inhibitor) attenuated the carvacrol-induced collagen production, SB203580 (a specific p38 inhibitor) had no inhibitory effect on it (Fig. 5A and B). Consistent with this result, the carvacrol-induced activation of JNK and ERK1/2 was also inhibited by PAO (Fig. 6).

4. Discussion

In this report, we investigated the collagen production-inducing effect and the molecular mechanism by which carvacrol elicits its activating effects on collagen expression in human dermal fibroblasts. Our data demonstrated that carvacrol up-regulates collagen expression, which strongly suggests that carvacrol ameliorates skin aging through the activation of collagen expression. Among the various pathways that are known to be involved in collagen expression, we found that carvacrol induces collagen expression by activating the AP-1 pathway (PLCγ1-Ca²⁺-JNK & ERK1/2). Notably, this is the first...
report that demonstrates the involvement of PLCγ1 in collagen expression.

Several important signaling pathways induce expression of the type I collagen gene; one of these is the SP-1-mediated pathway, the second is the Smad protein-mediated pathway, and the third is the AP-1 pathway. In our study to determine the mechanisms of action in the collagen production-inducing effect of carvacrol, carvacrol had no effect on Smad 2 phosphorylation and carvacrol-induced activation of COL1A2 promoter was not reduced by siRNA for SP-1. However, AP-1 promoter activation and phosphorylation of both JNK and ERK1/2 were induced by carvacrol. These results suggest that carvacrol induces collagen expression through the AP-1 activation pathway and, more specifically, through the activation of JNK and ERK1/2.

Intracellular signaling pathways such as phospholipase Cγ (PLCγ) and Ca²⁺ have been shown to be involved in the MAPK activation pathway [21]. These reports prompted us to investigate the specific upstream proteins that are involved in the carvacrol-induced phosphorylation of MAPK. Carvacrol induced both the mobilization of Ca²⁺ and the phosphorylation of PLCγ1. Type I collagen production was also attenuated by PAO, a PLCγ1 inhibitor, by PD98059 (a specific ERK1/2 inhibitor) and by SP600125 (a specific JNK inhibitor). This result sug-

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**Fig. 4** Both intracellular mobilization of Ca²⁺ and PLCγ1 phosphorylation were induced by carvacrol. (A) Human dermal fibroblasts were loaded with Fura2-AM for 60 min and then washed with Krebs-HEPES buffer for [Ca²⁺]i measurement. [Ca²⁺]i was measured fluorometrically every 5 s for 100 s after the addition of 50 μM carvacrol as described in Section 2. The curve shown is representative of three independent experiments. Cv: carvacrol. (B) Human dermal fibroblasts were pretreated with the indicated concentrations of carvacrol for 15 min. Western blot analysis of whole cell lysates was performed as described in Section 2. Changes in PLCγ1 phosphorylation were assessed. *p < 0.05 vs. untreated control.

**Fig. 5** Inhibition of the carvacrol-induced collagen production by PAO, PD98059, SP600125, and SB203580. (A and B) Effects of inhibitors on carvacrol-induced type I procollagen synthesis as determined using a sandwich immunoassay kit (TAKARA BIO, INC., Japan) (B). Data are expressed as means ± S.D. *p < 0.05 vs. control, **p < 0.05 vs. carvacrol-treated controls. Cv: carvacrol.
gests the involvement of PLCγ1 signaling (PLCγ1-Ca2+-ERK1/2/JNK) in the carvacrol-induced expression of the type I collagen gene. Here, we show that PLCγ1 signaling is involved in the carvacrol-induced expression of the type I collagen gene. However, this only applied to high concentrations of carvacrol (>20 μM). As shown in our results, although low concentrations of carvacrol (<10 μM) did not induce PLCγ1 phosphorylation and Ca2+ mobilization, low concentrations of carvacrol did induce the expression of the type I gene. In addition, at low concentration of carvacrol, Smad and SP-1 were not activated. Therefore, another molecular pathway may operate in the carvacrol-induced expression of type I collagen gene upon treatment with low concentrations of carvacrol (<10 μM). Further study is required to elucidate this pathway.

Chronic exposure of the skin to the sun is associated with multiple alterations in structure and function [22—24]. One of these changes is the rearrangement of collagen and elastic fibers in the extracellular matrix of the dermis. Many investigations have focused on the specific mechanisms involved in collagen production in order to develop new therapeutic agents for skin aging. Along these lines, only a few of the known agents are able to stimulate collagen production, including asiaticoside, retinoic acid, and sphingosine 1-phosphate [25—27]. Among them, retinoic acid is the only agent that has been developed as a pharmaceutical drug. Although the efficacy of retinoic acid has been demonstrated by both in vitro and in vivo assays, it has also been reported to have serious side effects [28]. Therefore, carvacrol, with its anti-wrinkle effect through the induction of collagen production, might be useful as a possible therapeutic agent or adjunctive agent to retinoic acid for the treatment of skin aging.

Taken together, the data from this study demonstrate that carvacrol induces collagen expression by activating intracellular Ca2+ mobilization via PLCγ1. Additionally, the fact that carvacrol activated AP-1 promoter reporter activity and phosphorylation of JNK and ERK1/2, but did not induce the phosphorylation of p38 MAPK, suggests the involvement of the PLCγ1-Ca2+-JNK/ERK1/2 signaling pathway in the carvacrol-induced expression of the type I collagen gene.

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References

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