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Diarylheptanoids suppress expression of leukocyte adhesion molecules on human vascular endothelial cells

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Abstract

Diarylheptanoids possess potent anti-inflammatory properties. However, the mechanism of their action is not fully understood. In this study, we found that three diarylheptanoids, 1-(3,5-dimethoxy-4-hydroxyphenyl)-7-phenylhept-1-en-3-one (YPE-01), yakuchinone B and demethyl-yakuchinone B, reduced the adhesion of both human monocytic cell line U937 and human eosinophilic cell line EoL-1 cells to tumor necrosis factor-α (TNF-α)-treated human umbilical vein endothelial cells. In addition, they suppressed interleukin-1β- or TNF-α-induced expression of E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on the surface of the endothelial cells. Since YPE-01 reduced both VCAM-1 and ICAM-1 mRNA induction in TNF-α-stimulated endothelial cells, diarylheptanoids appeared to suppress adhesion molecule expression at the transcriptional level. Furthermore, YPE-01 suppressed both VCAM-1 and ICAM-1 mRNA induction as well as edema in 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-inflamed mice ears in vivo. These results suggest that the anti-inflammatory action of diarylheptanoids is, at least in part, due to their suppressive effect on the surface expression of inducible adhesion molecules in endothelial cells, and subsequent leukocyte adhesion.

Keywords: Diarylheptanoid; Leukocyte adhesion molecule; Vascular endothelial cell; Anti-inflammation; 5-Lipoxygenase

1. Introduction

The adhesion of circulating leukocytes to the vascular endothelium and their migration into tissues at sites of inflammation are essential events in various inflammation processes. These processes are mediated by the expression of leukocyte adhesion molecules on the surface of vascular endothelial cells (Butcher, 1991; Carlos and Harlan, 1994; Springer, 1994). Major adhesion molecules are E-selectin (Bevilacqua et al., 1987), vascular cell adhesion molecule-1 (VCAM-1) (Osborn et al., 1989) and intercellular adhesion molecule-1 (ICAM-1) (Staunton et al., 1988), whose expression on endothelial cells is induced by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (Haraldsen et al., 1996). To reduce leukocyte adhesion to endothelial cells mediated by these adhesion molecules, various strategies have been used, such as the use of blocking antibodies for adhesion molecules (Barton et al., 1989), soluble forms of adhesion molecules (Gamble et al., 1990), synthetic peptide analogs based on the sequence of adhesion molecules (Fecondo et al., 1991), and agents that inhibit the upregulation of adhesion molecules (Boschelli et al., 1995).

It has been reported that phenolic diarylheptanoids such as yakuchinone B and demethyl-yakuchinone B derivatives of yakuchinones isolated from the fruit of *Alpinia oxyphylla* Miguel (Zingiberaceae), inhibit 5-lipoxygenase and cyclooxygenase (Flynn and Rafferty, 1986; Iwakami et al., 1986; Kiuchi et al., 1992). Our recent study demonstrated that a novel phenolic diarylheptanoid derivative, 1-(3,5-di-methoxy-4-hydroxyphenyl)-7-phenylhept-1-en-3-one (YPE-01), inhibited 5-lipoxygenase and suppressed arachidonic acid- and 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced ear edema in mice (Yamazaki et al., 1998). The presence of a phenolic group in the diarylheptanoids is essential for the inhibition of 5-lipoxygenase and cyclooxygenase (Kiuchi et al., 1992). However, it has been reported that nonphenolic diarylheptanoids also have anti-

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inflammatory effects in various experimental models of inflammation in vivo (Claeson et al., 1993, 1996). Therefore, the mechanism of action of diarylheptanoids is not fully understood.

Since the adhesion of leukocytes to the vascular endothelium is an important step in inflammation, we hypothesized that the diarylheptanoids may exert part of their anti-inflammatory effects via suppression of the adhesion of leukocytes to endothelial cells. In the present study, we investigated the effects of several dialylheptanoids on the adhesion of leukocytes to human umbilical vein endothelial cells and the expression of leukocyte adhesion molecules on endothelial cells.

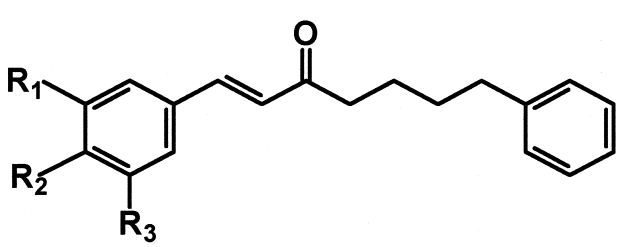
2. Materials and methods

2.1. Materials

YPE-01, 1-(4-hydroxyphenyl-3-methoxy)-7-phenylhept-1-en-3-one (yakuchinone B), 1-(3,4-dihydroxyphenyl)-7-phenylhept-1-en-3-one (demethyl-yakuchinone B) and 1-(3,4,5-trimethoxy)-7-phenylhept-1-en-3-one (trimethoxy-YPE-01) (Fig. 1) were chemically synthesized by Yakult (Tokyo, Japan). Indomethacin and nordihydroguaiaretic acid were purchased from Sigma (St. Louis, MO). Other materials were purchased from the following sources: RPMI1640, TNF-α and TPA (Sigma); interleukin-1β (Genzyme, Cambridge, MA); Dulbecco's phosphate-buffered saline (−) (PBS; Nissui Pharmaceutical, Tokyo, Japan); fetal calf serum and bovine serum albumin fraction V (Boehringer Mannheim, Mannheim, Germany); and calcein acetomethylester (Molecular Probes, Eugene, OR).

2.2. Cells and cell cultures

Human umbilical vein endothelial cells were purchased from Cell Systems (Kirkland, WA) and cultured in CS-C complete medium (Cell Systems) using collagen type I-coated culture dishes (Iwaki, Chiba, Japan) at 37°C in 5% CO_2. Human monocytic leukemia cell line U937 and human eosinophilic leukemia cell line EoL-1 were obtained from American-Type Culture Collection (Rockville, MD) and Riken Cell Bank (Ibaraki, Japan), respectively, and cultured in RPMI1640 supplemented with 10% (v/v) fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, Grand Island, NY) at 37°C in 5% CO_2.



I: R_1=OCH_3, R_2=OH, R_3=OCH_3
II: R_1=OCH_3, R_2=OH, R_3=H
III: R_1=OH, R_2=OH, R_3=H
IV: R_1=OCH_3, R_2=OCH_3, R_3=OCH_3

Fig. 1. The structures of diarylheptanoids. I, YPE-01; II, yakuchinone B; III, demethyl-yakuchinone B; IV, trimethoxy-YPE-01.

2.3. Animals

ICR mice (male, 7 weeks of age) were purchased from Japan SLC (Shizuoka, Japan). The animal experimentation guidelines of our institute were followed.

2.4. Adhesion assay

The adhesion assays were performed as previously described with modifications (De Clerck et al., 1994; Braut-Boucher et al., 1995). When human umbilical vein endothelial cells reached confluence on collagen type I-coated 96-well culture plates (Iwaki), they were first pretreated with the drugs for 2 h, then coincubated with both the drugs and TNF-α (50 U/ml) for 6 h in RPMI1640 containing 5% (v/v) fetal calf serum at 37°C in 5% CO_2. Before addition of U937 or EoL-1 cells to the endothelial cells, U937 or EoL-1 cells (5×10^6 cells/ml) were labeled for 40 min at 37°C with the fluorescence dye calcein acetomethylester (5 μM) in RPMI1640 containing 5% (v/v) fetal calf serum. The cells were then washed two times with, and resuspended in (final concentration, 1×10^6 cells/ml), RPMI1640 containing 5% (v/v) fetal calf serum. The media containing the labeled U937 or EoL-1 cells (100 μl/well) were added to the endothelial cells in 96-well plates from which the drugs and TNF-α were removed, and incubated for 1 h at 37°C. The plates were then washed two times with RPMI1640, and PBS (100 μl/well) was added. The relative fluorescence was determined using a fluorescence microplate reader, Titertek Fluoroskan II (Flow Laboratories, McLean, VA), with excitation and emission wavelengths of 485 and 538 nm, respectively. Adherent cell numbers were calculated by comparing fluorescence to standard curves of calcein acetomethylester activity/cell.

2.5. Measurement of cell adhesion molecule expression

E-selectin, VCAM-1 and ICAM-1 expression on the surface of human umbilical vein endothelial cells was analyzed by cellular enzyme-linked immunosorbent assay (cell ELISA). The cell ELISA was performed as previously described with modifications (Lee et al., 1997).

When human umbilical vein endothelial cells reached confluence on 96-well culture plates, they were first pretreated with the drugs for 2 h, and then coincubated with both the drugs and TNF-α (50 U/ml) or interleukin-1β (100 U/ml) for 6 h (E-selectin) or for 8 h (VCAM-1 and ICAM-1) in RPMI1640 containing 5% (v/v) fetal calf serum at 37°C in 5% CO_2. The cells were washed once with PBS and then fixed with 0.25% (v/v) glutaraldehyde in PBS for 5 min at 4°C. Fixed cells were washed three times with PBS and blocked with PBS containing 0.1% (w/v) bovine serum albumin and 100 mM glycine overnight at 4°C. The blocked cells were washed with PBS containing 0.05% (w/v) Tween 20 and then incubated with 0.17 μg/ml anti-human E-selectin (clone 68-5H11, PharMingen, San Diego, CA), 0.25 μg/ml anti-human VCAM-1 (clone 51-10C9, PharMingen) or 0.05 μg/ml anti-human ICAM-1 mouse monoclonal antibody (clone HA58, PharMingen), which was diluted with PBS containing 0.1% (w/v) bovine serum albumin. After 1 h at room temperature, the cells were washed two times with PBS containing 0.05% (w/v) Tween 20 and then incubated with 0.08 μg/ml peroxidase-conjugated anti-mouse IgG Fab fragments (Jackson Immuno Research Laboratories, West Grove, PA) diluted with PBS containing 0.1% (w/v) bovine serum albumin for 1 h at room temperature. At the end of incubation, the cells were washed three times with PBS containing 0.05% (w/v) Tween 20 and incubated with 100 μl/well of substrate solution (citrate buffer pH 5.6 containing 0.1 mg/ml *o*-phenylenediamine and 0.015% (v/v) H_2O_2). After 30 min, 50 μl/well of 4 N H_2SO_4 was added to stop the reaction, and absorbance at 492 nm was measured using SPECTRA max™ 250 (Molecular Devices, Sunnyvale, CA). The results are expressed as optical density (OD) values.

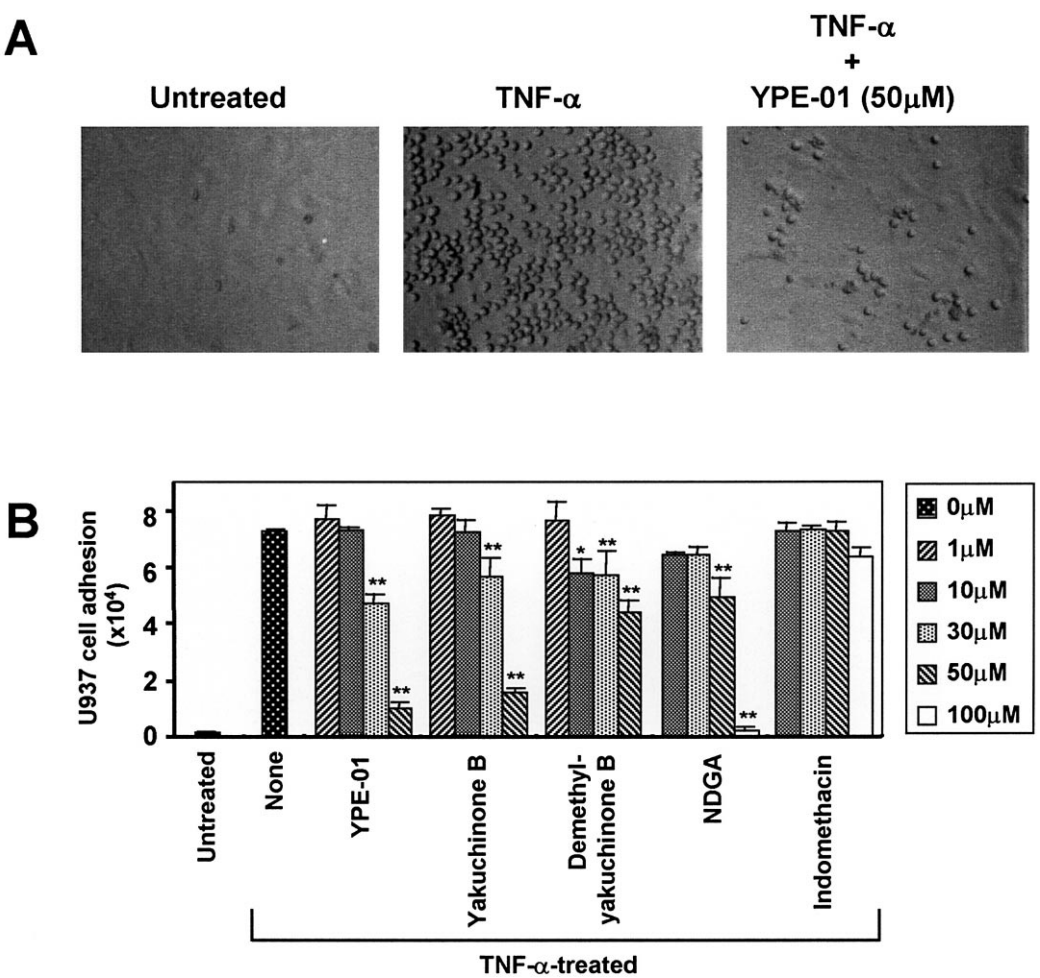


Fig. 2. Effects of diarylheptanoids on U937 cell adhesion to TNF-α-treated human umbilical vein endothelial cells. Confluent human umbilical vein endothelial cells were first pretreated with the drugs for 2 h and then coincubated with both the drugs and TNF-α (50 U/ml) for 6 h. U937 cell adhesion was measured by the adhesion assay as described in Materials and methods. (A) Microphotographs of U937 cells adhered to untreated, TNF-α-treated, and YPE-01 and TNF-α-cotreated human umbilical vein endothelial cells. (B) Dose-dependent effects of diarylheptanoids, nordihydroguaiaretic acid and indomethacin on U937 cell adhesion to TNF-α-treated human umbilical vein endothelial cells. Data are means ± S.D. of triplicate cultures. Results are representative of three independent experiments. $^{*}P < 0.01$ and $^{**}P < 0.001$ vs. TNF-α-treated control cells. NDGA, nordihydroguaiaretic acid.

2.6. Cell viability

The viability of human umbilical vein endothelial cells treated with various drugs was determined with the Cyto-Tox 96® Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI), according to the manufacturer's instructions.

2.7. Reverse transcription polymerase chain reaction

VCAM-1 and ICAM-1 mRNA levels in human umbilical vein endothelial cells or mouse ear were analyzed by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted using Isogen (Nippon Gene, Tokyo, Japan) from the endothelial cells or the ears of mice. The cDNA synthesis and PCR amplification reactions were done using RT-PCR high-Plus- (TOYOBO, Osaka, Japan) according to the manufacturer's instructions. The PCR primers for mouse VCAM-1 (Watanabe et al., 1995) and ICAM-1 (Oran et al., 1997) were synthesized by Nippon Flour Mills (Kanagawa, Japan). The sequences of the primers were as follows: mouse VCAM-1 sense = 5′-CTCTGTACATCCCTCCACA-3′, mouse VCAM-1 antisense = 5′-GGGACTGTGCAGTTGACAG-3′, mouse ICAM-1 sense = 5′-TCGGAGGATCACAAACGAAGC-3′, mouse ICAM-1 antisense = 5′-AACATAAGA-GGCTGCCATCACG-3′. The PCR protocol using the synthesized primers was 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, for 35 cycles. The PCR primers for mouse glyceraldehyde 3-phosphate dehydrogenase (G3PDH) were purchased from Clontech Laboratories (Palo Alto, CA). The PCR primers for human β-actin, ICAM-1 and VCAM-1 were purchased from R&D Systems (Minneapolis, MN). The PCR using the purchased primers was done according to each manufacturer's instructions. The PCR products were analyzed by electrophoresis using 2% agarose gels and were visualized by ethidium bromide staining and UV illumination.

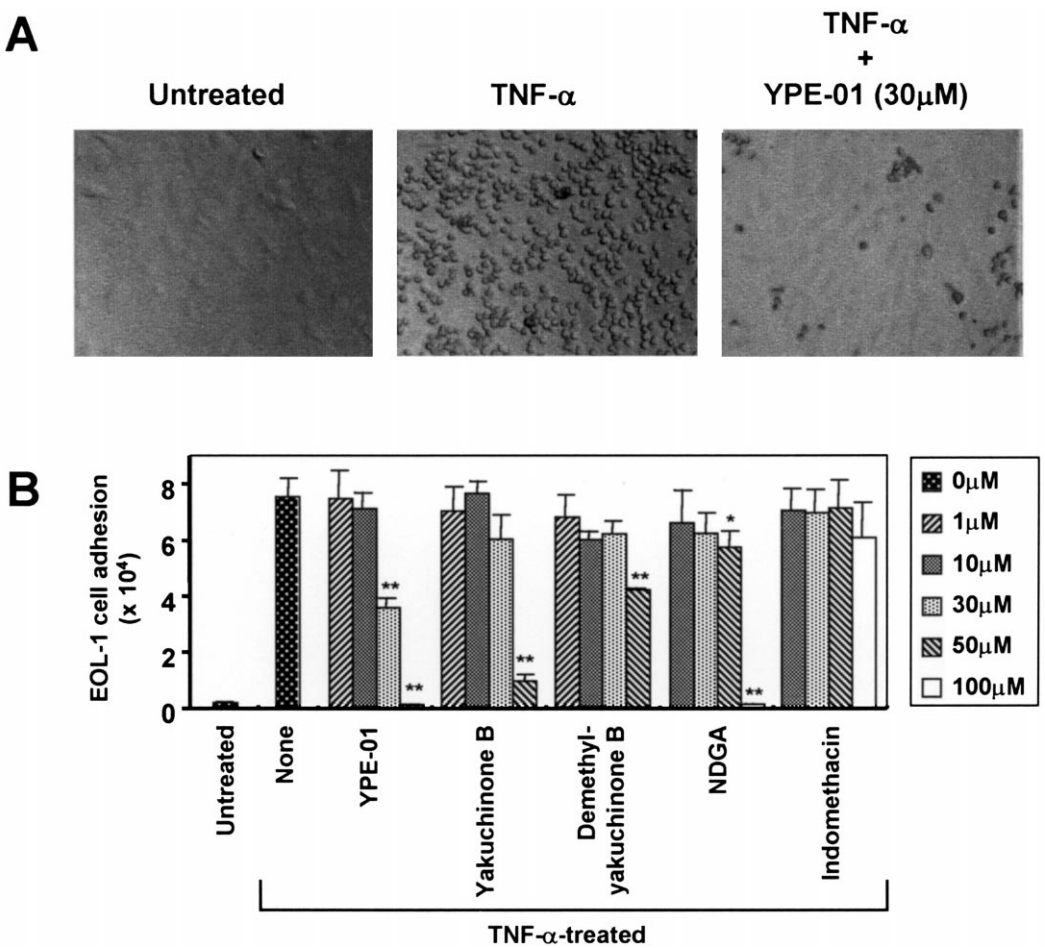


Fig. 3. Effects of diarylheptanoids on EoL-1 cell adhesion to TNF-α-treated human umbilical vein endothelial cells. Confluent human umbilical vein endothelial cells were first pretreated with the drugs for 2 h and then coincubated with both the drugs and TNF-α (50 U/ml) for 6 h. EoL-1 cell adhesion was measured by the adhesion assay as described in Materials and methods. (A) Microphotographs of EoL-1 cells adhered to untreated, TNF-α-treated, and YPE-01 and TNF-α-cotreated human umbilical vein endothelial cells. (B) Dose-dependent effects of diarylheptanoids, nordihydroguaiaretic acid and indomethacin on EoL-1 cell adhesion to TNF-α-treated human umbilical vein endothelial cells. Data are means ± S.D. of triplicate cultures. Results are representative of three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.001$ vs. TNF-α-treated control cells. NDGA, nordihydroguaiaretic acid.

2.8. TPA-induced ear edema

TPA was dissolved in acetone at a concentration of 40 μg/ml and applied in a volume of 20 μl to both the inner and outer surfaces of the right ears of ICR mice. At the same time, the drugs were similarly applied as solutions in acetone. The ear swelling was assessed by measuring the right ear thickness using an Upright Dial Gauge (Ozaki, Tokyo, Japan).

2.9. Statistical analysis

The data are expressed as means ± S.D. Statistical analysis was done using Dunnett's test. P values less than 0.05 were considered to be significant.

3. Results

3.1. Effects of diarylheptanoids on leukocyte adhesion to TNF-α-treated human umbilical vein endothelial cells

Since human U937 monocytic cells express lymphocyte-function associated antigen-1 (LFA-1), very late antigen-4 (VLA-4) and sialylated Lewis x glycoprotein (sLex), which are ligands for ICAM-1, VCAM-1 and E-selectin, respectively, they have been used in adhesion experiments with human umbilical vein endothelial cells (Hauser et al., 1993). Human EoL-1 eosinophilic cells also constitutively express high levels of LFA-1 and VLA-4 (Jung et al., 1994). We examined the effects of diarylheptanoids, YPE-01, yakuchinone B and demethyl-yakuchinone B on the

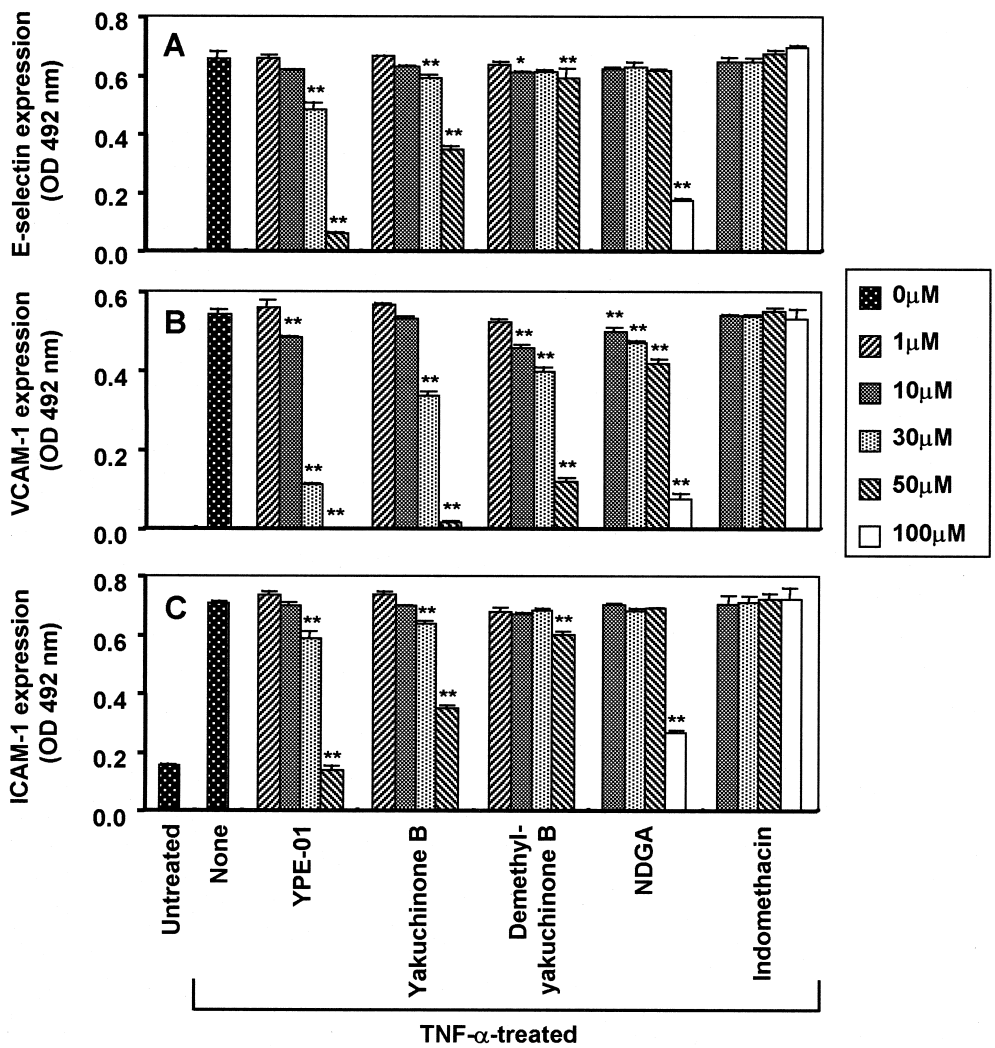


Fig. 4. Effects of diarylheptanoids on TNF-α-induced cell-surface expression of (A) E-selectin, (B) VCAM-1 and (C) ICAM-1 in human umbilical vein endothelial cells. Confluent human umbilical vein endothelial cells were first pretreated with the drugs for 2 h and then coincubated with both the drugs and TNF-α (50 U/ml) for 6 h (E-selectin) or 8 h (VCAM-1 and ICAM-1). Leukocyte adhesion molecule expression on the surface of endothelial cells was analyzed by cell ELISA as described in Materials and methods. Data are means ± S.D. of triplicate cultures. Results are representative of three independent experiments. $^{*}P < 0.01$ and $^{**}P < 0.001$ vs. TNF-α-treated control cells. NDGA, nordihydroguaiaretic acid.

adhesion of U937 (Fig. 2) and EoL-1 (Fig. 3) cells to TNF-α-treated human umbilical vein endothelial cells. U937 and EoL-1 cell adhesion to untreated endothelial cells was negligible. However, stimulation of the endothelial cells by TNF-α for 6 h markedly enhanced the adhesion of both cells. The maximal U937 and EoL-1 cell adhesion induced by TNF-α was concentration dependently suppressed by YPE-01, with IC_{50} values of 36.1 and 29.0 μM, respectively. In addition, yakuchinone B and demethyl-yakuchinone B suppressed the TNF-α-induced adhesion. Nordihydroguaiaretic acid, a 5-lipoxygenase inhibitor (Bokoch and Reed, 1981), also suppressed the TNF-α-induced U937 and EoL-1 cell adhesion to the endothelial cells, with IC_{50} values of 64.2 and 67.2 μM, respectively, whereas indomethacin, a cyclooxygenase inhibitor (Vane, 1971), had no effect at concentrations up to 100 μM.

3.2. Effects of diarylheptanoids on TNF-α or interleukin-1β-induced expression of leukocyte adhesion molecules

To explore whether diarylheptanoids modulate the cytokine-induced expression of leukocyte adhesion molecules, E-selectin, VCAM-1, and ICAM-1 protein expression on the surface of TNF-α (Fig. 4) or interleukin-1β (Fig. 5)-treated human umbilical vein endothelial cells was examined by cell ELISA. Although ICAM-1 was constitutively expressed on cytokine-untreated endothelial cells, expression of E-selectin and VCAM-1 was not detected. Expression of these molecules was significantly increased

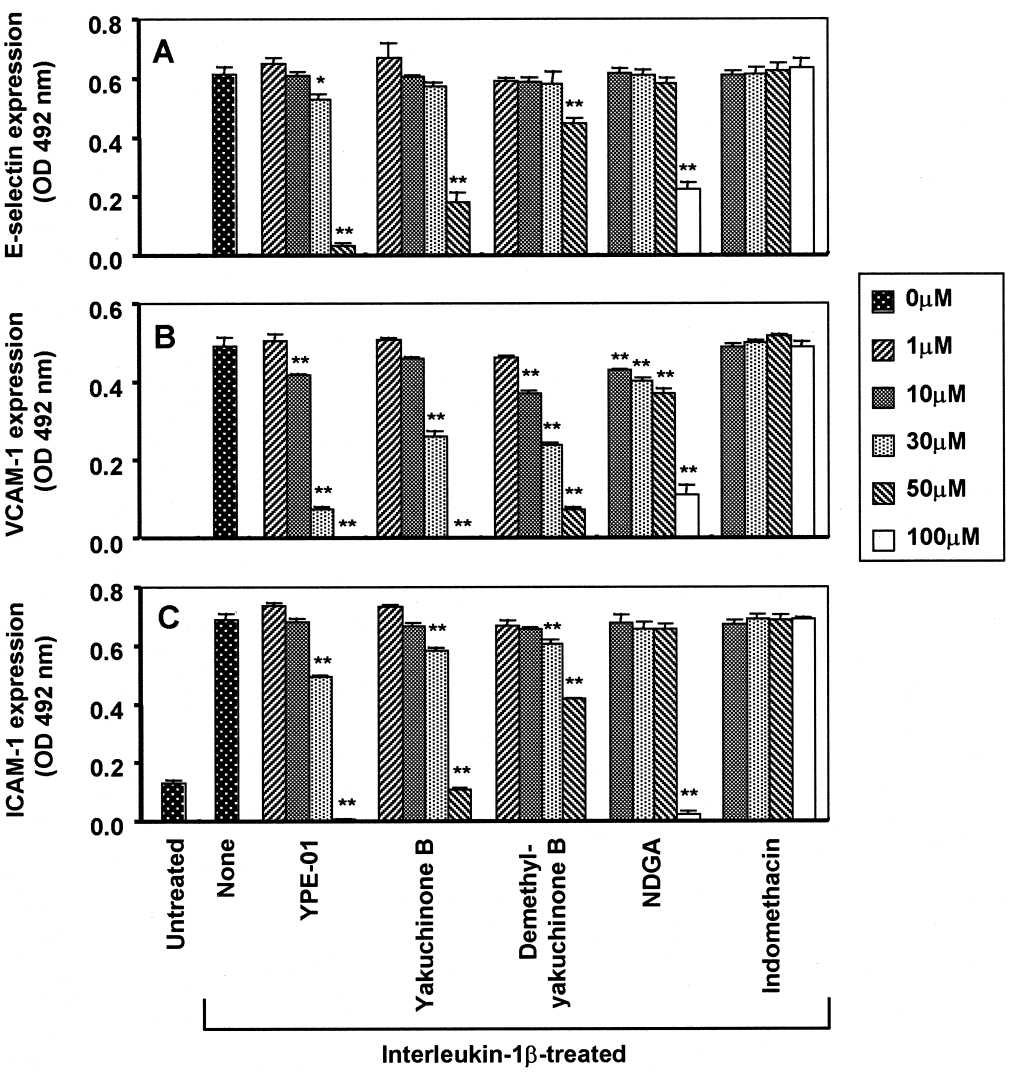


Fig. 5. Effects of diarylheptanoids on interleukin-1β-induced cell-surface expression of (A) E-selectin, (B) VCAM-1 and (C) ICAM-1 in human umbilical vein endothelial cells. Confluent human umbilical vein endothelial cells were first pretreated with the drugs for 2 h and then coincubated with both the drugs and interleukin-1β (100 U/ml) for 6 h (E-selectin) or 8 h (VCAM-1 and ICAM-1). Leukocyte adhesion molecule expression on the surface of endothelial cells was analyzed by cell ELISA as described in Materials and methods. Data are means ± S.D. of triplicate cultures. Results are representative of three independent experiments. $^{*}P < 0.01$ and $^{**}P < 0.001$ vs. interleukin-1β-treated control cells. NDGA, nordihydroguaiaretic acid.

by TNF-α or interleukin-1β. As shown in Fig. 4, YPE-01 suppressed TNF-α-induced E-selectin, VCAM-1 and ICAM-1 expression on the endothelial cell surface in a concentration-dependent manner, with IC_{50} values of 37.4, 21.4 and 40.3 μM, respectively. A similar suppression by YPE-01 of E-selectin, VCAM-1 and ICAM-1 expression was observed when the endothelial cells were treated with interleukin-1β (Fig. 5). In addition, yakuchinone B and demethyl-yakuchinone B suppressed TNF-α or interleukin-1β-induced cell adhesion molecule expression. The suppressive effects of these diarylheptanoids were somewhat preferential for VCAM-1 expression. Nordihydroguaiaretic acid also suppressed the TNF-α or interleukin-1β-induced expression of cell adhesion molecules, but less potently than diarylheptanoids. In contrast, indomethacin had no effect on cytokine-induced expression of adhesion molecules at concentrations up to 100 μM. Treatment of human umbilical vein endothelial cells with these drugs did not affect cell viability.

Furthermore, we examined the effects of trimethoxy-YPE-01, a nonphenolic diarylheptanpid, on the expression of leukocyte adhesion molecules on human umbilical vein endothelial cells. Trimethoxy-YPE-01 had no inhibitory effect on 5-lipoxygenase activity (IC_{50}: > 100 μM) in contrast to YPE-01 (IC_{50}: 0.28 μM) (Yamazaki et al., 1998). However, trimethoxy-YPE-01 also suppressed TNF-α-induced E-selectin, VCAM-1 and ICAM-1 expression, similar to the effect of YPE-01, with IC_{50} values of 30.5, 17.8 and 39.9 μM, respectively.

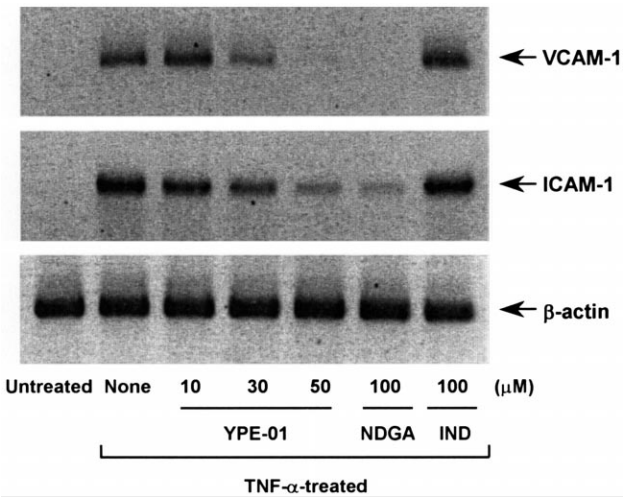


Fig. 7. Effects of YPE-01 on TNF-α-induced VCAM-1 and ICAM-1 mRNA expression in human umbilical vein endothelial cells. Confluent human umbilical vein endothelial cells were treated with drugs for 2 h before stimulation with TNF-α (50 U/ml) for 5 h. Total RNA was extracted and subjected to RT-PCR using specific primers for human VCAM-1, ICAM-1 and β-actin as described in Materials and methods. Results are representative of three independent experiments. NDGA, nordihydroguaiaretic acid; IND, indomethacin.

For time course studies, YPE-01 and trimethoxy-YPE-01 were added before and after TNF-α stimulation (Fig. 6). The suppressive effects of YPE-01 and trimethoxy-YPE-01 on TNF-α-induced expression of VCAM-1 weakened to

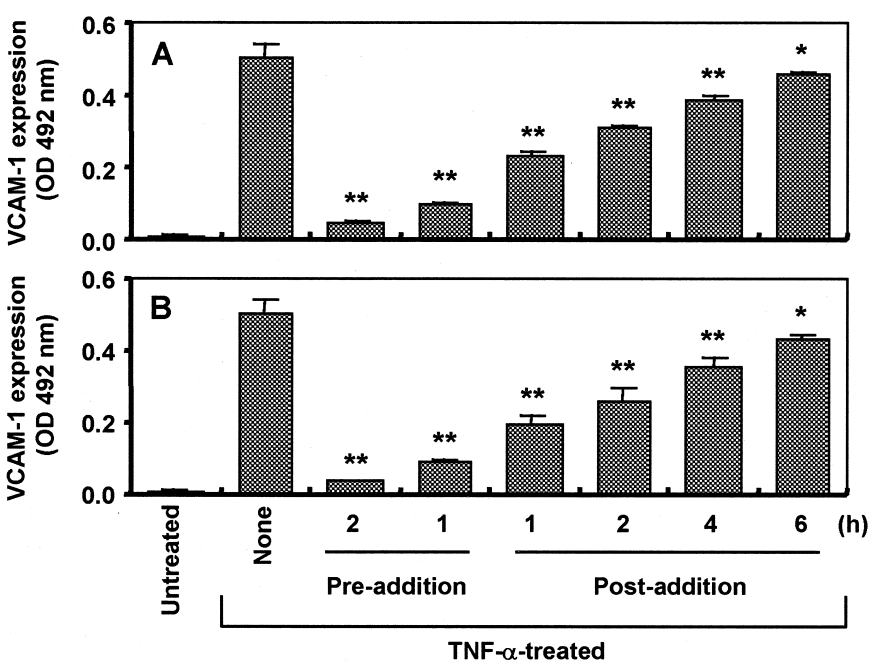


Fig. 6. Time dependence of effects of diarylheptanoids on TNF-α-induced cell surface expression of VCAM-1 in human umbilical vein endothelial cells. YPE-01 (30 μM, A) or trimethoxy-YPE-01 (30 μM, B) was added at various times before or after addition of TNF-α (50 U/ml) to human umbilical vein endothelial cells. VCAM-1 expression on the endothelial cells was analyzed 8 h after addition of TNF-α by cell ELISA as described in Materials and methods. Data are means ± S.D. of triplicate cultures. Results are representative of three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.001$ vs. TNF-α-treated control cells.

the same extent in a time-dependent manner after stimulation with TNF-α. However, the suppressive effects were detectable when YPE-01 or trimethoxy-YPE-01 was added 6 h after stimulation with TNF-α.

3.3. Effects of YPE-01 on TNF-α-induced expression of leukocyte adhesion molecule mRNA in human umbilical vein endothelial cells

We examined whether the decrease of leukocyte adhesion molecule expression on the surface of TNF-α-stimulated human umbilical vein endothelial cells caused by diarylheptanoids was secondary to changes in mRNA levels using RT-PCR (Fig. 7). In untreated endothelial cells, VCAM-1 and ICAM-1 mRNAs were not detected, but TNF-α significantly increased these mRNA levels. YPE-01 markedly suppressed the TNF-α-induced expression of VCAM-1 and ICAM-1 mRNAs. Nordihydroguaiaretic acid also suppressed the TNF-α-induced expression of VCAM-1 and ICAM-1 mRNAs. In contrast, indomethacin had no effect on the TNF-α-induced expression of these mRNAs.

3.4. Effects of YPE-01 on expression of leukocyte adhesion molecule mRNAs in TPA-inflamed ears of mice

To assess the effect of diarylheptanoids on the expression of cell adhesion molecules in vivo, we studied VCAM-1 and ICAM-1 mRNA expression in TPA-induced mouse ear edema (Fig. 8). The expression of VCAM-1 and ICAM-1 mRNAs, which was not detected in untreated mice ears, was significantly increased in the ears, as was the edematous reaction, by topical application of TPA. YPE-01 markedly suppressed the TPA-induced expression of VCAM-1 and ICAM-1 mRNAs as well as ear edema.

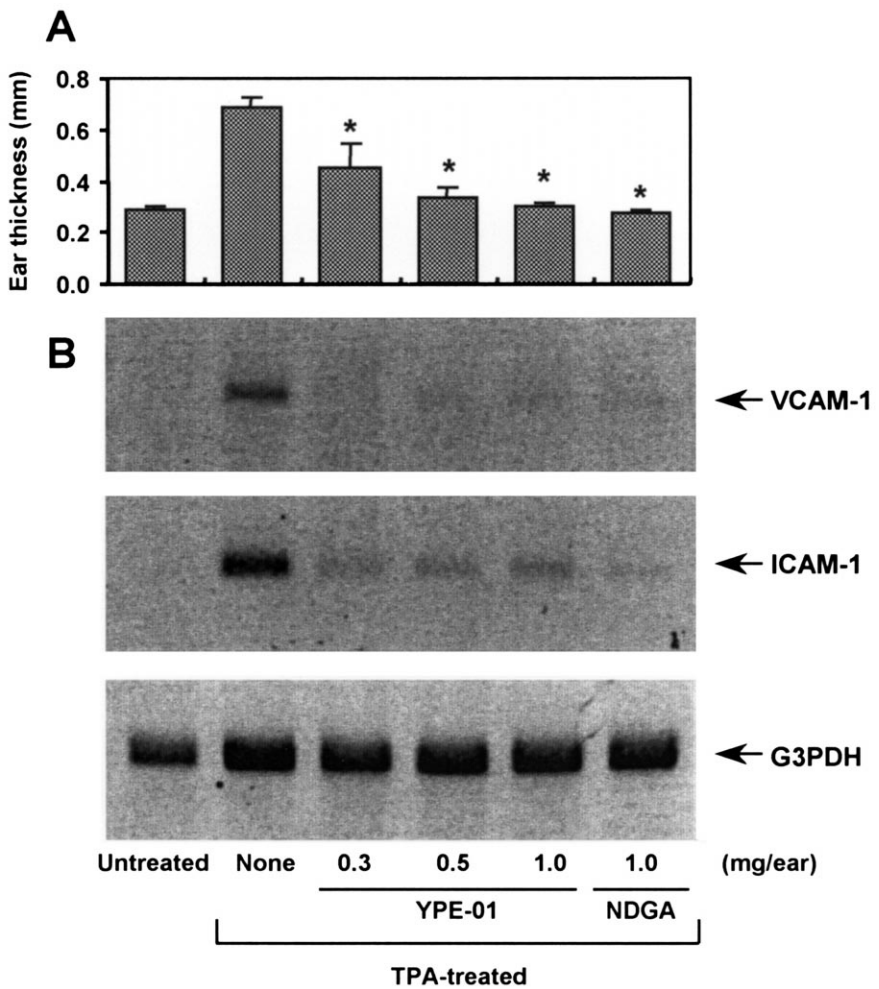


Fig. 8. Effects of YPE-01 on TPA-induced ear edema. YPE-01 was applied to the right ears of mice at the same time as TPA (0.8 μg/ear). (A) The ear thickness was measured 4 h after application of TPA. Data are means ± S.D. for six animals. Results are representative of three independent experiments. * $P < 0.001$ vs. TPA-treated control. (B) Total RNA was extracted from the ears of mice 4 h after application of TPA and subjected to RT-PCR using specific primers for mouse VCAM-1, ICAM-1 and G3PDH as described in Materials and methods. Results are representative of three independent experiments. NDGA, nordihydroguaiaretic acid.

Nordihydroguaiaretic acid also suppressed the expression of these mRNAs and ear edema.

4. Discussion

It is well established that inhibitors of 5-lipoxygenase, inhibitors of 5-lipoxygenase activating protein and inhibitors of cyclooxygenase have anti-inflammatory properties (Ford-Hutchinson et al., 1994; Vane and Botting 1996). Therefore, most studies of the anti-inflammatory action of diarylheptanoids have focused on their inhibitory effects on 5-lypoxygenase and cyclooxygenase (Flynn and Rafferty, 1986; Iwakami et al., 1986; Kiuchi et al., 1992; Yamazaki et al., 1998). The diarylheptanoids examined in this study, Yakuchinone B and demethyl-yakuchinone B, inhibit both 5-lipoxygenase and cyclooxygenase (Flynn and Rafferty, 1986; Iwakami et al., 1986; Kiuchi et al., 1992), and YPE-01 selectively inhibits 5-lipoxygenase (Yamazaki et al., 1998). The presence of a phenolic group in the chemical structure of the diarylheptanoids is essential for the inhibition of 5-lipoxygenase and cyclooxygenase (Kiuchi et al., 1992). However, it has been reported that nonphenolic diarylheptanoids also have anti-inflammatory effects on various experimental models of inflammation in vivo (Claeson et al., 1993, 1996). Accordingly, previous observations cannot sufficiently explain the mechanism by which diarylheptanoids exert their anti-inflammatory effects.

In the present study, we found that YPE-01, yakuchinone B and demethyl-yakuchinone B suppressed the adhesion of U937 and EoL-1 cells to TNF-α-treated human umbilical vein endothelial cells. These effects paralleled the decrease in surface expression of E-selectin, VCAM-1 and ICAM-1 in TNF-α- or interleukin-1β-treated human umbilical vein endothelial cells caused by these diarylheptanoids. Therefore, the diarylheptanoids may suppress leukocyte adhesion to endothelial cells through a reduction in leukocyte adhesion molecule expression on the surface of endothelial cells.

TPA-induced ear edema is a well-characterized experimental model of inflammation (Young et al., 1983; Carlson et al., 1985). Our recent study demonstrated that YPE-01 suppressed TPA-induced ear edema through the inhibition of 5-lipoxygenase (Yamazaki et al., 1998). It has been reported that TPA can induce the expression of E-selectin, VCAM-1 and ICAM-1 on the surface of human umbilical vein endothelial cells (Lane et al., 1989; Deisher et al., 1993), and the infiltration of inflammatory cells is a characteristic feature of TPA-induced ear edema (Young et al., 1983; Sánchez and Moreno, 1999). These findings suggest that the mechanism of action of diarylheptanoids on TPA-induced ear edema involves the suppression of adhesion molecule expression as well as 5-lipoxygenase activity. Therefore, we attempted to identify the in vivo effects of YPE-01 on the expression of VCAM-1 and ICAM-1 using TPA-induced ear edema. Topical application of TPA to ears of mice induced the expression of VCAM-1 and ICAM-1 mRNAs along with the edematous reaction in the ears. YPE-01 was found to suppress the TPA-induced expression of the mRNAs of these adhesion molecules as well as the ear edema. Thus, YPE-01 may exert its anti-inflammatory effect through not only the inhibition of 5-lipoxygenase, but also the suppression of leukocyte adhesion molecule expression.

YPE-01 reduced the TNF-α-induced expression of VCAM-1 and ICAM-1 proteins and mRNAs to a similar extent, suggesting that it regulates their expression at the transcriptional level. Cytokine-induced gene transcription of adhesion molecules in human umbilical vein endothelial cells is regulated by an antioxidant-sensitive mechanism, involving nuclear factor-κB (NF-κB) activation (Marui et al., 1993; Faruqi et al., 1997). Antioxidants, such as pyrrolidinedithiocarbamate, prevent NF-κB activation through inhibition of the proteolysis of the inhibitor of NF-κBα (IκB-α) (Henkel et al., 1993), followed by suppression of VCAM-1 expression on human umbilical vein endothelial cells. YPE-01 also has a phenolic group that could enable it to act as an antioxidant. However, YPE-01 had no inhibitory effects on TNF-α-induced proteolysis of IκB-α in human umbilical vein endothelial cells at concentrations up to 50 μM (data not shown). Thus, it appears that the mechanism of action of YPE-01 is different from that of antioxidants.

The mechanism by which the diarylheptanoids act on the expression of adhesion molecules is not fully understood. In this respect, the previous observation that 5-lipoxygenase inhibitors block agonist-induced adhesion molecule gene expression in human umbilical vein endothelial cells is of interest (Zhou et al., 1996; Lee et al., 1997). We also demonstrated that nordihydroguaiaretic acid, a well-characterized inhibitor of 5-lipoxygenase (Bokoch and Reed, 1981), suppressed the expression of VCAM-1 and ICAM-1 mRNAs in TNF-α-treated human umbilical vein endothelial cells in vitro and in the TPA-inflamed ears of mice in vivo. The phenolic diarylheptanoids, YPE-01, yakuchinone B and demethyl-yakuchinone B, inhibit 5-lipoxygenase with IC_{50} values of 0.28, 0.37 and 0.22 μM, respectively (Yamazaki et al., 1998). However, there was no correlation between the effects of diarylheptanoids on cell adhesion molecule expression and 5-lipoxygenase activity, because trimethoxy-YPE-01, a nonphenolic diarylheptanoid, which had no inhibitory effect on 5-lipoxygenase (IC_{50}: > 100 μM), also suppressed the TNF-α-induced expression of adhesion molecules on the surface of human umbilical vein endothelial cells, similar to the effect of YPE-01. In addition, indomethacin, a cyclooxygenase inhibitor (Vane, 1971), had no suppressive effect on the cytokine-induced expression of adhesion molecule proteins and mRNAs. Taken together, these results indicate that the diarylheptanoids suppress the expression of adhesion molecules through a

mechanism that is independent of their 5-lipoxygenase and cyclooxygenase inhibitory activity, but which remains to be clarified.

In conclusion, we have demonstrated that the diarylheptanoids suppress leukocyte adhesion to cytokine-stimulated endothelial cells through a reduction in E-selection, VCAM-1 and ICAM-1 expression on the surface of endothelial cells. Therefore, it is possible that the anti-inflammatory effects of the diarylheptanoids are, at least in part, due to their suppressive effect on the surface expression of inducible adhesion molecules in endothelial cells, and subsequent leukocyte adhesion, along with the known inhibitory action on 5-lipoxygenase and cyclooxygenase.

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