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Kujigamberol interferes with pro-inflammatory cytokine-induced expression of and N-glycan modifications to cell adhesion molecules at different stages in human umbilical vein endothelial cells

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ABSTRACT

Kujigamberol is the norlabdane compound isolated from Kuji amber and has recently been shown to prevent Ca^{2+}-signal transduction and exert anti-allergy effects *in vitro* and *in vivo*. However, the anti-inflammatory activities of kujigamberol remain unclear. In the present study, we investigated the biological activities of kujigamberol on cell adhesion molecules expressed on human umbilical vein endothelial cells (HUVEC) in response to pro-inflammatory cytokines. Kujigamberol decreased the molecular weight of intercellular adhesion molecule-1 (ICAM-1) by altering N-glycan modifications. In contrast to ICAM-1, kujigamberol reduced the interleukin-1α- or tumor necrosis factor α-induced expression of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin at the mRNA and protein levels. Kujigamberol B, but not kujiol A, decreased the molecular weight of the ICAM-1 protein. Kujigamberol moderately inhibited yeast α-glucosidases, whereas it was only weakly inhibited by kujigamberol B and more weakly by kujiol A. Three compounds did not inhibit Jack bean α-mannosidases. The present results reveal new biological activities of kujigamberol, which interfere with the pro-inflammatory cytokine-induced expression of and N-glycan modifications to cell adhesion molecules in HUVEC.

1. Introduction

Cell adhesion molecules are critical for regulating immune and inflammatory responses [1]. The integrin family members expressed on leukocytes bind to the members of the immunoglobulin superfamily expressed on endothelial cells, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [2, 3]. The expression of ICAM-1 and VCAM-1 are induced by pro-inflammatory cytokines, including interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) [4–6]. E-selectin belongs to a family of C-type lectins capable of binding to carbohydrate ligands [7, 8]. E-selectin is also up-regulated on endothelial cells by pro-inflammatory cytokines [4, 7]. ICAM-1, VCAM-1, and E-selectin play an essential role in the multistep process of leukocyte transmigration: E-selectin mediates the rolling of leukocytes on endothelial cells, while ICAM-1 and VCAM-1 are required for adhesion and transmigration [2, 3]. The transcription factor nuclear factor κB (NF-κB) signaling pathway is triggered by pro-inflammatory cytokines [9, 10]. NF-κB transcription factors regulate many of the genes that are necessary for immune and inflammatory responses, such as those encoding ICAM-1, VCAM-1, and E-selectin [4–6]. ICAM-1, VCAM-1, and E-selectin possess multiple potential glycosylation sites and undergo modifications to N-linked carbohydrate chains during their intracellular transport to the cell surface [11].

We identified a norlabdane compound (15,20-dinor-5,7,9-labdatrien-18-ol) named kujigamberol (Fig. 1A) from Kuji amber by screening using mutant yeast hypersensitive to Ca^{2+}-signal transduction and elucidated its absolute configuration by total synthesis [12, 13]. In addition to the growth-restoring activity of the mutant yeast, kujigamberol has been shown to inhibit the enzyme activity of glycogen synthase kinase 3β (GSK-3β) as well as to exert cytotoxicity against human leukemia HL-60 cells [12]. Kujigamberol has recently been shown to prevent degranulation and Ca^{2+} influx in rat basophilic leukemia RBL-2H3 cells and exert anti-allergic effects in a rhinitis model of guinea pigs [14]. Moreover, kujiol A (Fig. 10A) and kujigamberol B (Fig. 10A) have been isolated from Kuji amber as Ca^{2+}-signal transduction inhibitors [15]. These findings indicate that kujigamberol and its derivatives inhibit the Ca^{2+}-dependent signaling pathway in cells. However, the biological activities of kujigamberol on mammalian cells

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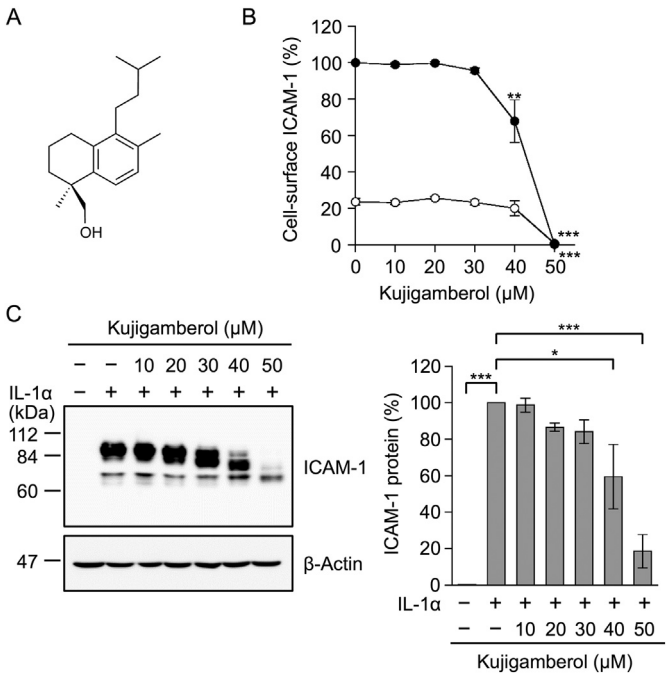

Fig. 1. Kujigamberol reduced IL-1α-induced ICAM-1 expression and decreased the molecular weight of ICAM-1 in HUVEC. (A) Structure of kujigamberol. (B) HUVEC were preincubated with or without kujigamberol for 1 h and then incubated with (filled circles) or without (open circles) IL-1α (1 ng/ml) for 6 h in the presence or absence of kujigamberol at the indicated final concentrations. The expression of cell-surface ICAM-1 was measured by cell-ELISA. ICAM-1 expression (%) is shown as the mean ± S.E. of three independent experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$, significantly different from kujigamberol (−). (C) HUVEC were preincubated with or without (−) kujigamberol for 1 h and then incubated with (+) or without (−) IL-1α (1 ng/ml) for 6 h in the presence or absence of kujigamberol at the indicated final concentrations. Cell lysates were analyzed by Western blotting. Data are representative of three independent experiments. The amounts of the ICAM-1 protein normalized to β-actin are shown as the mean ± S.E. of three independent experiments. $^{*}P < 0.05$ and $^{***}P < 0.001$, significantly different from IL-1α (+) kujigamberol (−).

have not yet been elucidated in detail.

ICAM-1 plays an essential role in immune and inflammatory responses [1–3]. Therefore, small-molecule compounds targeting cell-surface ICAM-1 are assumed to be candidates for anti-inflammatory agents and/or useful bioprobes for investigating intracellular processes [16]. In the course of our screening program, we found that kujigamberol reduced the molecular weight of ICAM-1 in human umbilical vein endothelial cells (HUVEC). In the present study, we investigated the biological effects of kujigamberol on cell adhesion molecules expressed on HUVEC in response to pro-inflammatory cytokines. We revealed that kujigamberol interferes with the pro-inflammatory cytokine-induced expression of and N-glycan modifications to cell adhesion molecules.

2. Materials and methods

2.1. Cells

HUVEC were obtained from Lonza (Walkersville, MD, USA) and cultured in Endothelial Cell Growth Medium 2 (PromoCell, Heidelberg, Germany) supplemented with penicillin-streptomycin antibiotic mixture (Nacalai Tesque, Kyoto, Japan). Human lung adenocarcinoma A549 cells (JCRB0076), human breast adenocarcinoma MCF-7 cells (JCRB0134), and human fibrosarcoma HT-1080 cells (JCRB9113) were provided by the National Institutes of Biomedical Innovation, Health and Nutrition JCRB Bank (Osaka, Japan). A549, MCF-7, and HT-1080 cells were cultured in RPMI 1640 medium (Thermo Fisher Scientific, Grand Island, NY, USA) supplemented with heat-inactivated fetal calf serum (Nichirei Bioscience, Tokyo, Japan) and penicillin-streptomycin mixed solution.

2.2. Reagents

Kujigamberol, kujiol A, and kujigamberol B were prepared as described previously [12, 15]. The HPLC analysis showed that purified preparations of kujigamberol, kujiol A, and kujigamberol B had purities of nearly 100% and were free of detectable amounts of endotoxins (Fig. S1). Human IL-1α and human TNF-α were kindly provided by Dainippon Pharmaceutical (Osaka, Japan). Brefeldin A (Merck, Darmstadt, Germany), castanospermine (Wako Pure Chemical Industries, Osaka, Japan), 1-deoxymannojirimycin (Santa Cruz Biotechnology, Dallas, TX, USA), 1-deoxynojirimycin (Wako Pure Chemical Industries), swainsonine (Cayman Chemical, Ann Arbor, MI, USA), tunicamycin (Wako Pure Chemical Industries), and *Z*-Val-Ala-Asp(OMe)-CH_2F (Z-VAD-FMK; Peptide Institute, Osaka, Japan) were commercially obtained.

2.3. Cell-ELISA

Cell-surface ICAM-1 was measured by cell-ELISA as described previously [17]. Cell-surface ICAM-1 was detected with a mouse anti-ICAM-1 antibody (clone 15.2; Leinco Technology Inc., St. Louis, MO, USA) as the primary antibody and a horseradish peroxidase (HRP)-conjugated anti-mouse IgG (H + L) antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) as the second antibody. After the colorimetric reaction, absorbance at 415 nm or 450 nm was measured by a Model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) or iMark™ microplate reader (Bio-Rad Laboratories).

2.4. MTT assay

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay was described previously [18]. HUVEC were pulsed with MTT for 4 h. Human cancer cell lines were pulsed with MTT for 2 h. Cells were incubated overnight in the presence of 5% SDS. Absorbance at 570 nm was measured by a Model 680 microplate reader or iMark™ microplate reader.

2.5. Crystal violet assay

The crystal violet assay was performed as described previously [19]. HUVEC were stained with crystal violet. Crystal violet was extracted with methanol, and absorbance at 570 nm was measured using the