Inhibitory Activity of Lignan Components from the Flower Buds of *Magnoliae fargesii* on the Expression of Cell Adhesion Molecules

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Inhibitory activity of lignans isolated from *Magnoliae fargesii* Cheng on cell adhesion molecules on the surface of THP-1 human monocytic cell lines were investigated. Among 16 lignan components tested, six displayed relatively potent inhibitory activity on the expression of both intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).

Key words intercellular adhesion molecule-1; vascular cell adhesion molecule-1; *Magnoliae fargesii*; lignan

Intercellular adhesion is an important early step in the complex but coordinated sequence of events leading to acute and chronic inflammatory diseases. Intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily of adhesion molecules, is basically expressed on the cell surface of endothelial cells, where its level can be increased during inflammatory conditions.[1,2] Cytokines such as INF-γ, IL-1β, and TNF-α can induce ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) on monocytes,[3,4] and it was reported that dexamethasone and cortisol markedly inhibited the expression of ICAM-1 on endothelial cells caused by endotoxin.[5,6]

The dried flower buds of *Magnolia fargesii* Cheng (Magnoliaceae) have been used to treat nasal empyema with headache, sinusitis, and allergic rhinitis.[7] Various components have been isolated from *M. fargesii*: oils, lignans, or sesquiterpenes having antihistamic, anticomplementary, and Ca^{2+}-antagonistic activty.[8,9] Recently platelet activating factor (PAF)-antagonistic activity of two lignans, magnone A and magnone B, was reported.[10] In this paper we report the inhibitory activity of lignans from *M. fargesii* on cell adhesion molecules of THP-1 cells.

MATERIALS AND METHODS

Materials Lignans were previously isolated from *M. fargesii*.[8,10] THP-1 cells were obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.). RPMI-1640, fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin) were from Gibco-BRL (Grand Island, NY, U.S.A.).

Cell Maintenance THP-1 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% antibiotics complex (penicillin and streptomycin). Cells were cultured on T-flasks in a CO_2 incubator supplying 5% CO_2 and 95% humid air at 37 °C and subcultured every 3 d for at least 2 weeks before the treatment of samples.

Cytotoxicity Assay Before the cell-based ELISA, evaluation of the untoxic range of samples was required. To determine the cytotoxicity of samples on THP-1 cells, 200 μl of cell suspension (5×10^4 cells/ml) was initially added to each well of a 96-well plate and incubated in the incubator at 37 °C for 1 h. After 10 μl of samples were treated, the plates were further cultured for 16 h. Final DMSO concentration was adjusted to 0.5% in all samples. Viability of cells was determined indirectly using Alama-Blue™ (Serotec, U.K.). Briefly, after the exposure of cells to test agents, each well was added with 50 μl Alama-Blue solution followed by incubation for 3 h in the incubator at 37 °C. Finally absorbance was measured at a wavelength of 570 nm, and background absorbance at 600 nm was subtracted.

Antibodies Monoclonal antibodies were obtained from the culture soup of hybridomas obtained from the American Type Culture Collection. Mouse IgG2a monoclonal antibody that reacts with human ICAM-1 was secreted by R6′5′D6′E9′B2 cells (ATCC HB-9580, mouse hybridoma) during 3 d of culture. For the purification of IgG type antibodies, the supernatant was loaded on an affinity column packed with protein G-sepharose 4B resin. After washing with PBS, the bound antibody was eluted with 0.1 M sodium citrate buffer (pH 3.5). In the same way, monoclonal antibody that reacted with human VCAM-1 was produced by VIII-6G10 cells (ATCC HB-10519, mouse hybridoma) and purified as the above.

Cell-Based ELISA To determine the expression of cell adhesion molecules, 5×10^4 of THP-1 cells were suspended in 200 μl of RPMI-1640 medium supplemented with 10% fetal bovine serum and plated in wells of a 96-well plate. After sample treatment (10 μl/well) and incubation in a CO_2 incubator for 1 h, cells were treated with TNF-α (10 ng/well) in order to induce the expression of adhesion molecules. Cytokine-treated cells were incubated for 16 h in a 37 °C CO_2 incubator. ICAM-1 and VCAM-1 were evaluated by Cell-ELISA (cell-based enzyme-linked immunoadsorbent assay) using anti-human monoclonal antibodies against ICAM-1 and VCAM-1, respectively. Cell-ELISA was carried out first by fixing THP-1 cells on the surface of a 96-well plate with a final concentration of 0.25% glutaraldehyde for 2 h. After washing 5 times with phosphate buffered saline-Tween 20 (PBST), the cell-fixed plates were blocked with 3% skim milk for 2 h and washed. Treatment with monoclonal antibody against ICAM-1 or VCAM-1 was administered for 2 h and the plate was washed. Anti-mouse IgG peroxidase conjugate was then treated for 2 h and washed again. Enzyme reaction was performed by adding 200 μl substrate solution (*O*-phenylene-diamine dihydrochloride 0.4 mg/ml, urea H_2O_2 0.4 mg/ml, phosphate–citrate buffer 0.05 M) for 5—10 min and then stopped with 3 M HCl. Colorimetric detection was conducted by reading at 490 nm.

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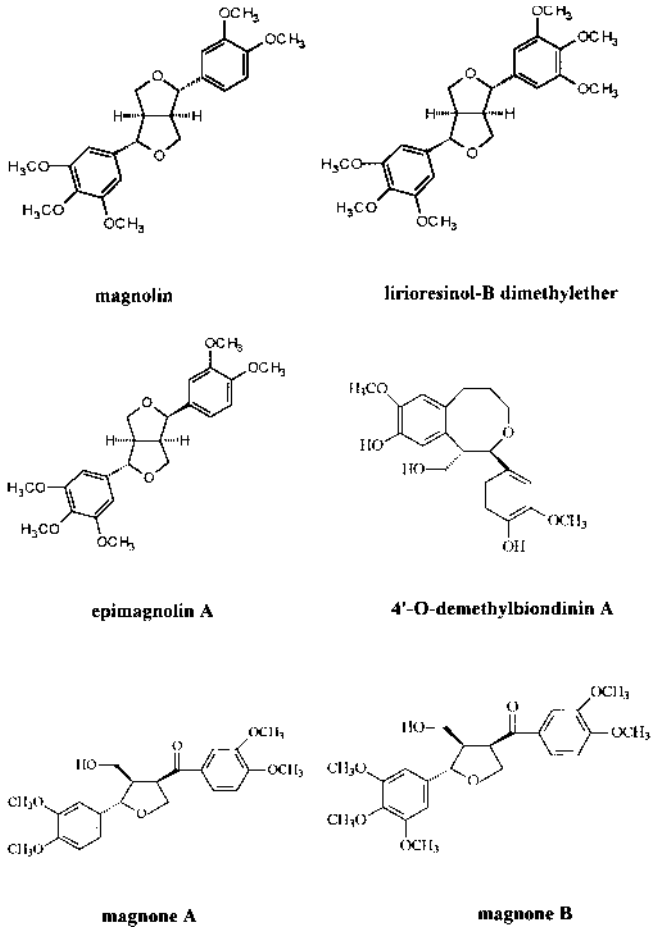

Fig. 1. Bioactive Lignans from the Flower Buds of *Magnolia fargesii*

RESULTS AND DISCUSSION

This report evaluates the inhibitory activity of lignans on the expression of cell adhesion molecules on THP-1 cells. In this assay system, both ICAM-1 and VCAM-1 were fully activated and expressed on the surface of these cells by treatment of 10 ng TNF-α in a well, and suppressed appropriately by 30 M dexamethasone as a positive control. Among the 16 tested lignan components isolated from *M. fargesii*, six lignans (Fig. 1): magnolin, lirioresinol-B dimethylether, epimagnolin, 4′-*O*-demethylbiondinin A, magnone A, and magnone B displayed relatively strong inhibitory activities of ICAM-1 expression induced by TNF-α. Similarly, the inhibitory activities of those lignans on VCAM-1 expression were also potent (Fig. 2). Among them, the activity of 4′-*O*-demethylbiondinin A and magnone A were relatively strong but less than that of dexamethasone. On the other hand, the other lignans such as biondinin B, 8′-hydro-7″-hydromagnofargesin, (+)-fargesol, biondinin A, biondinin E, fargesol methylester, demethylaschantin, aschantin, fargesin, and pinoresinol dimethylether, displayed a little effect. Most of the active lignans isolated from *M. fargesii* were not significantly cytotoxic even at 100 μM, which was the maximal concentration in our test, though magnone A, magnolin, and epimagnolin were more and less cytotoxic at that concentration (data not shown). No significant morphological change of the THP-1 cells by lignans of noncytotoxic concentrations were shown when the cells were treated with or without TNF-α of

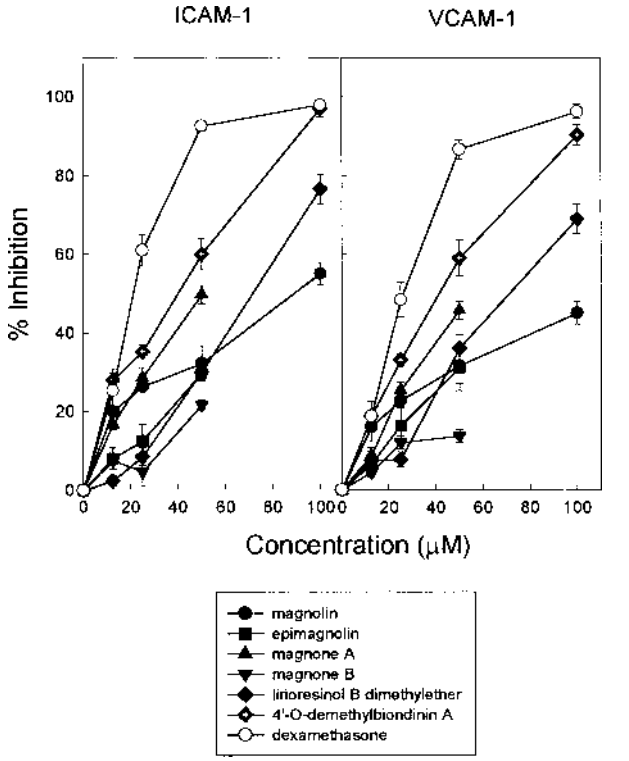

Fig. 2. Inhibitory Activity of Lignans on the Expression of Cell Adhesion Molecules on TNF-α-activated THP-1 Cells

10 ng/ml.

Anti-inflammatory activities of lignans have been reported, *e.g.* anti-allergic properties of yangambin,[11] a natural PAF antagonist, PAF receptor antagonism of a semisynthetic lignan[12] and TNF-α production by lignans from the rhizomes of *Coptis japonica* in LPS-stimulated RAW264.7 cells.[13]

We have studied the modulation of expression of cell adhesion molecules, another important target for the treatment of inflammation-related diseases, in endothelial cells and leukocytes by natural products. Intercellular adhesion is an important early step in the complex but coordinated sequence of events leading to acute and chronic inflammatory diseases. In this work six lignans isolated from *M. fargesii* are reported as inhibitors of the expression of the cell adhesion molecules ICAM-1 and VCAM-1. It was recently reported that magnone A and magnone B displayed anti-PAF activity[10] and magnolin and that lirioresinol B dimethylether had TNF-α suppressing activity in LPS-stimulated macrophages[14] from *M. fargesii.* Additionally anti-PAF activity of magnolin from *Magnolia biondii* Pamp was reported.[15] In view of these results, it is probably indicated that these bioactive lignans are compounds worth developing as novel anti-inflammatory drugs.

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