Effects of Oleanane-Type Triterpenoids from Fabaceous Plants on the Expression of ICAM-1

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We examined the inhibitory effects of oleanane-type triterpenoids from fabaceous plants on the TNF-α-induced expression of cell adhesion molecules on THP-1 human monocytic leukemia cells and compared them with a glucocorticoid dexamethasone. In a cell-based ELISA, abrisapogenol E, soyasapogenols B and C, soyasapogenol B, kuzusapogenol B-methyl ester, and oleanolic acid significantly inhibited intercellular adhesion molecule (ICAM-1) expression. Moreover, these triterpenoids showed the same activity as dexamethasone. On the other hand, the absence of hydroxyl group at C-24 position of sapogenin rather to increase ICAM-1 expression compared with the untreated control. We concluded that the activity of oleanane saponins and sapogenins against ICAM-1 expression are dependent upon the position of the hydroxyl group, and in particular upon the status of the C-21 and C-24 positions and of the glycosyl group at C-3 position.

Key words oleanane-type triterpenoid; fabaceous plant; intercellular adhesion molecule

Intercellular adhesion molecule-1 (ICAM-1) is members of the immunoglobulin superfamily of adhesion molecules, and appears to lead to acute and chronic inflammatory diseases. Recently we reported on the inhibitory activity of stilbenes on the TNF-α-induced overexpression of ICAM-1 and vascular cell adhesion molecule (VCAM-1) on the surfaces of THP-1 cells.[1] We now report on the inhibitory activities of oleanane-type triterpenes recently isolated from fabaceous plants[2] as a result of collaborative research group study on the cell adhesion molecules of THP-1 cells.

Oleanane-type triterpenes could be described as an olean-12-ene type triterpenes with a C-17 methyl group in this paper, and their glycosidic derivatives, the oleanane glucuronides, which include soyasaponin, and glycyrrhizin, are widely distributed in fabaceous plants and have known biological activities, for example, anti-hepatitis,[3] anti-hypercholesteremia,[4] anti-urolithiasis,[5] and anti-complementary activity.[6] On the other hand, limited biological activities have been reported for the oleanane-type sapogenols, except the hepatoprotective activity of soyasapogenol A or B.[7,8]

In the assay system used, ICAM-1 expressions on the surface of THP-1 cells were dramatically increased when the cells were activated by TNF-α, these were suppressed appropriately, and dexamethasone was used as a positive control.

MATERIALS AND METHODS

Isolation of Oleanane-Type Saponins and Sapogenols Oleanane-type saponins and sapogenols have been isolated from many fabaceous plants.[2]

Cell Culture THP-1 cells were obtained from the American Tissue Culture Collection (Rocksville, 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.). THP-1 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, penicillin (100000 units), and streptomycin (100 mg). Cells were cultured in T-flasks in a CO_2 incubator supplying 5% CO_2 and 95% humid air atmosphere at 37 °C and subcultured every 3 d for at least 2 weeks before treatment.

Cytotoxic Activity Before cell-based ELISA, the non-toxic range of samples was found. To determine the cytotoxic activities of the samples against THP-1 cells, 200 μl of cell suspension (5×10^4 cells/ml) was added to each well of a 96-well plate and incubated in a CO_2 incubator at 37 °C for 1 h. Ten microliters of sample solution was then added, and the cells were further cultured for 16 h. The final dimethyl sulfoxide (DMSO) concentration was adjusted to 0.5% in all tests. Cell viabilities were determined indirectly using AlamaBlue™ (Serotec, U.K.).

Preparation of Monoclonal Antibodies against Adhesion Molecules Monoclonal antibodies were obtained from hybridoma culture soup described previously.[1] Briefly, mouse IgG2a monoclonal antibody that reacts with human ICAM-1 was secreted by R6′5′D6′E9′B2 cells (ATCC HB-9580, mouse hybridoma) over 3 d of culture. To purify the IgG type antibodies, the supernatant was loaded onto an affinity column packed with protein G-Sepharose 4B resin. After washing with phosphate buffered saline (PBS), the bound antibody was eluted with 0.1 M sodium citrate buffer (pH 3.5). The monoclonal antibodies did not display cross-reactivity with other cell surface molecules and specifically bound plate-coated ICAM-1 in a dose-dependent manner.

Determination of Adhesion Molecules To determine the expression of cell adhesion molecules, 5×10^4 THP-1 cells were suspended in 200 μl of RPMI-1640 medium supplemented with 10% FBS and plated on a well of a 96-well plate. After sample treatment (10 μl/well) and incubation in a CO_2 incubator for 1 h, the 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 was evaluated using a cell-based enzyme-linked immunoadsorbent assay (Cell-ELISA) using monoclonal antibodies against human ICAM-1. Cell-ELISA was carried out as described previously.[1]

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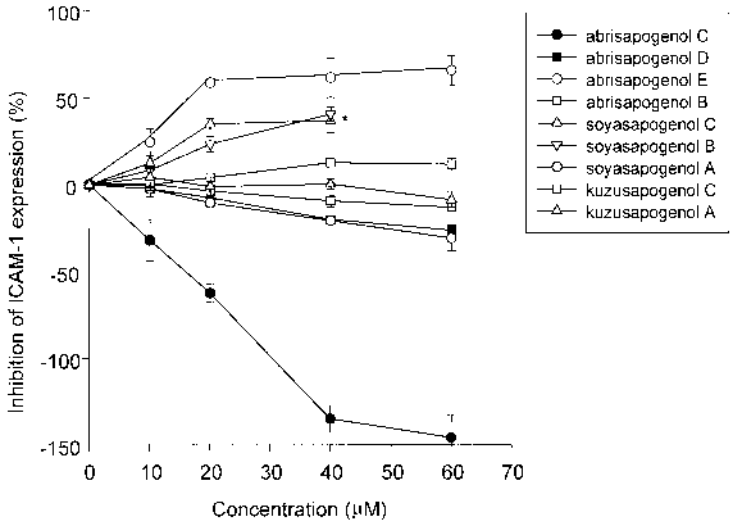
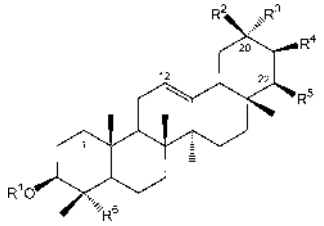


* The data of soyasapogenols B and C at 60 μM concentration were not able to estimate because of their cytotoxicity.

**Symbols opened, Group I; Symbols closed, Group II; Symbols shaded, Group III

Fig. 1. Effect of Oleanane-Type Sapogenins on Inhibition of ICAM-1 Expression on the THP-1 Cells

Table 1. Chemical Structures and the Relationship between the Structure and the Inhibitory Activity of Oleanane-Type Triterpenes on ICAM-1 Expression



	R^1	R^2	R^3	R^4	R^5	R^6	Activity
Sapogenins							
Group I							
Abrisapogenol C	H	CH_2OH	CH_3	OH	OH	H	Strong activation
Abrisapogenol D	H	CH_3	CH_2OH	H	OH	H	Mild activation
Group II							
Abrisapogenol B	H	CH_2OH	CH_3	H	OH	CH_2OH	Mild inhibition
Abrisapogenol E	H	CH_3	CH_2OH	H	OH	CH_2OH	Strong inhibition
Soyasapogenol B	H	CH_3	CH_3	H	OH	CH_2OH	Strong inhibition
Soyasapogenol C[a)]	H	CH_3	CH_3	H	H	CH_2OH	Strong inhibition
Group III							
Kuzusapogenol A	H	CH_2OH	CH_3	OH	OH	CH_2OH	No effect
Kuzusapogenol B-Me	H	COOMe	CH_3	OH	OH	CH_2OH	Not detected[c)]
Kuzusapogenol C	H	CH_3	CH_3	OH	H	CH_2OH	Mild activation
Soyasapogenol A	H	CH_3	CH_3	OH	OH	CH_2OH	Mild activation
Saponins (Group IV)							
Abrisaponin A	Fab[b)]	H	CH_2OH	CH_3	OH	H	Mild inhibition
Abrisaponin SB	Fab	CH_3	CH_3	H	OH	CH_2OH	Mild inhibition
Robinioside E	Fab	CH_2OH	CH_3	H	OH	CH_2OH	Mild inhibition
Sophoraflavoside II	Fab	COOH	CH_3	H	OH	CH_2OH	Mild inhibition
Soyasaponin A_3	Fab	CH_3	CH_3	OH	OH	CH_2OH	Mild inhibition
Soyasapogenol B monoglucuronide	GlcA	CH_3	CH_3	H	OH	CH_2OH	No effect
Subproside V	Fab	CH_3	CH_2OGlc	H	OH	CH_2OH	Mild inhibition
Wistariasaponin B_2	Fab	CH_3	CH_2OH	H	OH	CH_2OH	No effect

a) Soyasapogenol C have double bond between at C-21 and C-22. *b*) Fab (*β*-fabatriosyl): –glcA–gal–rha. *c*) Not detectable due to the cytotoxicity.

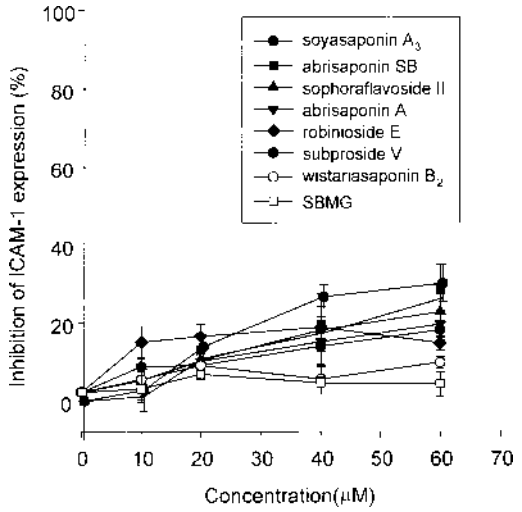


Fig. 2. Effect of Oleanane-Type Saponins on Inhibition of ICAM-1 Expression on the THP-1 Cells

RESULTS AND DISCUSSION

During the course of our studies on the inhibition of cell adhesion molecule expression, we found that some oleanane-type sapogenins and saponins from fabaceous plants display relatively potent activity. In a cell-ELISA, abrisapogenol E, soyasapogenols B and C all significantly inhibited ICAM-1 expression to the same extent as dexamethasone (39.5% inhibition at a 30 μM concentration), which was used as a positive control (Fig. 1). But most oleanane-type saponins were practically devoid of any significant activity (Fig. 2). Most sapogenins (Groups II, III) and saponins (Group IV) have common hydroxyl group (–OH) at C-24 position. However, the inhibitory activity of sapogenins and saponins was significantly decreased by a hydroxyl group at C-21 position (Group III). In case of saponins of Group IV (oleanane glucuronides), their mild inhibitory activity is probably due to a glycosyl moiety. On the other hand, sapogenins lacking a hydroxyl group at the C-24 position (Group I) rather significantly to increase ICAM-1 expression (negative values) relative to untreated control (Table 1).

Therefore, we concluded that inhibitory activity of oleanane saponins and sapogenins on ICAM-1 expression are dependent on the positions of the hydroxyl groups, in particular, the C-21 and C-24 positions and the glycosyl group at C-3. Similarly, it has been reported that the hepatoprotective activity of the oleanane-type saponins was enhanced by polar groups at the C-21 position but reduced by polar groups at C-20.[2)]

In addition to hepatoprotective activity, various other biological activities of oleanane-type saponins or sapogenins has been reported, including, antinephritic,[9)] anti-ulcerogenic,[10)] and anti-complementary activity,[6)] which are related to anti-inflammatory activity. Our findings suggest that oleanane-type saponins or sapogenols with specific side groups have potential as anti-inflammatory drugs.

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