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Asiatic Acid, Corosolic Acid, and Maslinic Acid Interfere with Intracellular Trafficking and N-Linked Glycosylation of Intercellular Adhesion Molecule-1

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The pentacyclic triterpenoid ursolic acid was previously shown to inhibit the intracellular trafficking of intercellular adhesion molecule-1 (ICAM-1) from the endoplasmic reticulum (ER) to the Golgi apparatus. In the present study, we further investigated the biological activities of three pentacyclic triterpenoids closely related to ursolic acid on the interleukin 1α-induced expression and intracellular trafficking of ICAM-1. In human lung adenocarcinoma A549 cells, asiatic acid, corosolic acid, and maslinic acid interfered with the intracellular transport of ICAM-1 to the cell surface. Endoglycosidase H-sensitive glycans were linked to ICAM-1 in asiatic acid-, corosolic acid-, and maslinic acid-treated cells. Unlike corosolic acid, asiatic acid and maslinic acid increased the amount of the ICAM-1 protein. Moreover, asiatic acid increased the colocalization of ICAM-1 with calnexin (an ER marker), but not GM130 (a cis-Golgi marker). Asiatic acid, corosolic acid, and maslinic acid inhibited yeast α-glucosidase activity, but not Jack bean α-mannosidase activity. These results indicate that asiatic acid, corosolic acid, and maslinic acid interfere with the intracellular transport of ICAM-1 to the cell surface and cause the accumulation of ICAM-1 linked to endoglycosidase H-sensitive glycans.

Key words asiatic acid; corosolic acid; maslinic acid; pentacyclic triterpenoid; intercellular adhesion molecule-1; intracellular trafficking

MATERIALS AND METHODS

Cell-surface adhesion molecules play an essential role in the regulation of immune and inflammatory responses.1 As one of the most important adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) is mainly up-regulated by the transcription factor nuclear factor κB (NF-κB) in response to inflammatory cytokines.2 ICAM-1 interacts with multiple molecules, including lymphocyte function-associated antigen-1 and Macrophage-1 antigen.3 As a post-translation modification, ICAM-1 is highly glycosylated with N-linked glycans.4 ICAM-1 is up-regulated on vascular endothelial cells during inflammatory responses and is required for the recruitment of leukocytes and their transmigration to inflammation sites.5,6 In addition, ICAM-1 has been reported to play a role in cancer metastasis.5

Pentacyclic triterpenoids are often found as secondary metabolites of plants.7,8 Ursolic acid, oleanolic acid, and betulinic acid are major pentacyclic triterpenoids, and have been reported to exhibit many biological activities, such as anti-inflammatory and anti-cancer activities.9–12 We previously reported that ursolic acid prevents the intracellular trafficking and causes the accumulation of ICAM-1 linked with high-mannose-type glycans in the endoplasmic reticulum (ER).13 In contrast, betulinic acid and oleanolic acid did not affect the intracellular transport of ICAM-1 to the cell surface, but interfered with the N-linked glycan modification of ICAM-1.14 These findings suggest that pentacyclic triterpenoids possess multiple targets in the intracellular trafficking of glycoproteins and their post-translational modifications.

Asiatic acid, corosolic acid, and maslinic acid (Fig. 1A) are pentacyclic triterpenoids closely related to ursolic acid, and have been shown to exhibit diverse biological activities, such as anti-inflammatory and anti-tumor activities.15–18 In the present study, we further investigated the biological activities of these pentacyclic triterpenoids on the inflammatory cytokine-induced expression and intracellular trafficking of as well as post-translational modifications to ICAM-1. We found that asiatic acid, corosolic acid, and maslinic acid interfered with the intracellular trafficking and caused the accumulation of ICAM-1 linked to endoglycosidase H (Endo H)-sensitive glycans. In addition, we showed that asiatic acid and maslinic acid, but not corosolic acid, increased the expression of the ICAM-1 protein.

Cell Culture Human lung adenocarcinoma A549 cells (JCRB 0076) were provided by the National Institutes of Biomedical Innovation, Health and Nutrition JCRB Cell Bank (Osaka, Japan). A549 cells were cultured in RPMI 1640 medium (Thermo Fisher Scientific, Grand Island, NY, U.S.A.) supplemented with heat-inactivated fetal calf serum (Nichirei Bioscience, Tokyo, Japan) and penicillin–streptomycin mixed solution (Nacalai Tesque, Kyoto, Japan).

Reagents Asiatic acid (Tokyo Chemical Industry, Tokyo, Japan), corosolic acid (Sigma-Aldrich, St. Louis, MO, U.S.A.; Abcam, Cambridge, U.K.), maslinic acid (Sigma-Aldrich), ursolic acid (Sigma-Aldrich), and 1-deoxymannojirimycin hydrochloride (Santa Cruz Biotechnology, Dallas, TX, U.S.A.)

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were commercially obtained. Human interleukin-1α (IL-1α) was kindly provided by Dainippon Pharmaceutical (Osaka, Japan). Human IL-1β was purchased from Genzyme Diagnostics (Cambridge, MA, U.S.A.).

**Cell-Enzyme-Linked Immunosorbent Assay (ELISA)**

Cell-ELISA was performed basically as described previously. A549 cells were washed twice with phosphate-buffered saline (PBS) and then fixed with 1% paraformaldehyde-PBS. Fixed cells were incubated serially with a mouse anti-ICAM-1 antibody (clone 15.2; Leinco Technologies, St. Louis, MO, U.S.A.) and horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin G (IgG) (H+L) antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, U.S.A.), followed by the HRP colorimetric reaction. Cell-surface ICAM-1 expression was evaluated by measuring absorbance at 415 or 450 nm.

**Flow Cytometry**

A549 cells were harvested and washed with PBS, followed by fixation with 1% paraformaldehyde-PBS. Fixed cells were stained with a mouse anti-ICAM-1 antibody (clone 15.2) or mouse IgG1 isotype control (MOPC-21; BioLegend, San Diego, CA, U.S.A.) for 1 h and then with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody (Jackson ImmunoResearch) for 30 min. Stained cells were analyzed using the Guava EasyCyte Plus™ System (Merck Millipore, Darmstadt, Germany).

**Cell Viability Assays**

Crystal violet staining and the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay were performed basically as described previously. Cells were incubated with MTT for the last 2 h of the incubation. Cells were washed with PBS and stained with crystal violet. Absorbance at 570 or 595 nm was measured.

**Western Blotting**

The preparation of cell lysates and Western blotting were performed as described previously. Cells were washed with PBS and lysed by 1% Triton X-100 lysis buffer. Cytoplasmic fractions were collected by centrifugation (15300×g, 5 min) as supernatants. Precipitates were washed twice with lysis buffer and prepared as nuclear fractions. Primary antibodies for β-actin (AC-15, Sigma-Aldrich; 2F3, Wako Pure Chemical Industries, Ltd., Osaka, Japan), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (6C5; Sigma-Aldrich; 19000×g, 10 min), and RelA (C-20; Santa Cruz Biotechnology), lamin A/C (E-1; Santa Cruz Biotechnology), and ICAM-1 (clone 28; BD Biosciences, San Jose, CA, U.S.A.) were used. Proteins were separated on 10% SDS-polyacrylamide gels and Western blotted using mouse anti-ICAM-1 antibody (clone 15.2; Leinco Technologies, St. Louis, MO, U.S.A.) or mouse IgG1 isotype control (MOPC-21; BioLegend, San Diego, CA, U.S.A.). Cells were heated at 100°C for 10 min and then incubated with or without Endo H or PNGase F for 1 h, followed by sodium decyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

**Confocal Microscopy**

Confocal microscopy was performed basically as described previously. Cells were grown on coverslips coated with Cellmatrix® type I-C (Nitta Gelatin, Osaka, Japan). Cells were fixed with 4% paraformaldehyde-PBS and washed with PBS. Fixed cells were stained with mouse anti-ICAM-1 antibody (clone 15.2) together with a rabbit anti-calnexin antibody (EPR3632, Abcam) or rabbit anti-GM130 antibody (EP892Y, Abcam), and then an Alexa Fluor 488-conjugated anti-mouse IgG antibody (A-11029; Thermo Fisher Scientific) or Alexa Fluor 594-conjugated anti-rabbit IgG antibody (A-11037; Thermo Fisher Scientific). Cells were observed with the confocal laser scanning microscope system FV 10i (Olympus, Tokyo, Japan). Regarding the quantification of the co-localization of ICAM-1 with calnexin or GM130, at least 50 cells of five different images per one experiment were evaluated.

**Glycosidase Assays**

Glycosidase assays were performed as described previously. Yeast α-glucosidase and Jack bean (Canavalia ensiformis) α-mannosidase were obtained from Oriental Yeast (Osaka, Japan) and Sigma-Aldrich, respectively. p-Nitrophenyl-α-D-glucopyranoside and 4-nitrophenyl-α-D-mannopyranoside were used for substrates of α-glucosidase and α-mannosidase, respectively. Absorbance at 405 nm was measured.

**Statistical Analysis**

The significance of differences was evaluated by a one-way ANOVA followed by Tukey’s test for multiple comparisons unless otherwise specified.

**RESULTS**

**Corosolic Acid and Maslinic Acid Inhibited the Expression of Cell-Surface ICAM-1 Induced by IL-1α**

In response to pro-inflammatory cytokines, vascular endothelial cells up-regulate cell-surface adhesion molecules, such as ICAM-1. We have used human lung adenocarcinoma A549 cells as a model cell line for vascular endothelial cells because they are responsive to multiple pro-inflammatory cytokines (e.g. tumor necrosis factor-α (TNF-α), IL-1α, and IL-1β) and express a detectable amount of cell-surface ICAM-1 by cell-ELISA when stimulated. We previously showed that ursoic acid inhibited the IL-1α-induced expression of cell-surface ICAM-1 in A549 cells and human umbilical vein endothelial cells. In order to investigate the inhibitory activities of pentacyclic triterpenoids structurally related to ursolic acid, A549 cells were preincubated with them for 1 h and then incubated with IL-1α for 6 h, followed by cell-ELISA to measure the expression of cell-surface ICAM-1. Asiatic acid did not markedly affect the expression of cell-surface ICAM-1 at concentrations up to 100 μM (Fig. 1B). Corosolic acid down-regulated the IL-1α-induced expression of cell-surface ICAM-1 in a dose-dependent manner and completely at 60 μM (Fig. 3C). Corosolic acid decreased the amount of cell-surface ICAM-1 at a concentration of 100 μM, while asiatic acid at 100 μM did not markedly affect the expression of cell-surface ICAM-1 (Figs. 2A, B).

MTT-reducing activity was used to evaluate the viability of A549 cells. Asiatic acid, corosolic acid, and maslinic acid did not decrease MTT-reducing activity at concentrations up to 100 μM (Figs. 3A–C). Crystal violet staining was then used to measure adherent cells. Asiatic acid only slightly decreased the number of adherent cells at 100 μM (Fig. 3D). In contrast, corosolic acid reduced the number of adherent cells in a dose-dependent manner (Fig. 3E). Maslinic acid did not affect the
number of adherent cells up to 80 µM and decreased it partially at 100 µM (Fig. 3F). These results indicate that asiatic acid only weakly affected the viability of A549 cells at concentrations up to 100 µM, while corosolic acid and maslinic acid preferentially inhibited the expression of cell-surface ICAM-1 more than reducing the number of adherent cells.

Asiatic Acid and Maslinic Acid Up-Regulated the Expression of the ICAM-1 Protein Induced by IL-1α and IL-1β A549 cells were preincubated with pentacyclic triterpenoids for 1 h and then incubated with IL-1α for 6 h. ICAM-1 was detected as multiple bands in IL-1α-stimulated 549 cells, possibly due to heterogeneous glycosylation. As a common feature, the molecular weight of ICAM-1 was decreased by asiatic acid, corosolic acid, and maslinic acid (Figs. 4A–C). Moreover, we found that asiatic acid and maslinic acid increased the amount of the ICAM-1 protein, particularly at 100 and 60 µM, respectively (Figs. 4A, C). In contrast, corosolic acid decreased the amount of the ICAM-1 protein at concentrations more than 80 µM (Fig. 4B). A quantitative comparison of the cell-surface ICAM-1 and ICAM-1 proteins indicated that asiatic acid, corosolic acid, and maslinic acid interfered with the intracellular transport of ICAM-1 to the cell surface.

IL-1α and IL-1β transmit intracellular signals via the IL-1 receptor.21) We previously showed that IL-1β up-regulated the expression of ICAM-1 in A549 cells.20) A549 cells were preincubated with pentacyclic triterpenoids for 1 h and then incubated with IL-1β for 6 h. In IL-1β-stimulated A549 cells, asiatic acid and maslinic acid increased the amount of the ICAM-1 protein, whereas it was decreased by corosolic acid (Fig. 5).

Asiatic Acid, Corosolic Acid and Maslinic Acid Exerted Different Effects on ICAM-1 Transcription Induced by IL-1α To evaluate the expression of ICAM-1 mRNA, A549 cells were preincubated with asiatic acid, corosolic acid, and maslinic acid for 1 h, and stimulated with IL-1α for 2 h, followed by the preparation of total RNA, cDNA synthesis, and real-time PCR. Asiatic acid only slightly increased IL-1α-induced ICAM-1 mRNA expression (Fig. 6A). Corosolic acid partially reduced IL-1α-induced ICAM-1 mRNA expression (Fig. 6B). In contrast, maslinic acid increased IL-1α-induced ICAM-1 expression.
ICAM-1 mRNA expression at 60–100 µM (Fig. 6C). These results indicated that asiatic acid, corosolic acid and maslinic acid exerted different effects on IL-1α-induced ICAM-1 transcription.

ICAM-1 is mainly regulated by the NF-κB transcription factor.2) To examine the effects of asiatic acid on the NF-κB signaling pathway, A549 cells were treated with asiatic acid for 1 h and then stimulated with IL-1α for 30 min, followed by Western blotting using an antibody specific to the NF-κB subunit RelA (also known as p65). Upon the IL-1α stimulation, a part of RelA was translocated from the cytoplasm to the nucleus (Fig. 7). Asiatic acid did not markedly affect the nuclear translocation of RelA and the amount of RelA in the cytoplasm at concentrations up to 100 µM (Fig. 7). These results are consistent with asiatic acid not greatly affecting IL-1α-induced ICAM-1 mRNA expression (Fig. 6A).

Endo H-Sensitive Glycans Were Mainly Linked to ICAM-1 in Asiatic Acid-, Corosolic Acid-, and Maslinic Acid-Treated Cells In order to investigate whether asiatic acid, corosolic acid, and maslinic acid affect N-linked glycan modifications, cell lysates were digested by PNGase F and Endo H. Major ICAM-1 bands migrated mostly at the same molecular weight on SDS-PAGE when A549 cells were treated with asiatic acid, corosolic acid, maslinic acid, and ursolic acid (Fig. 8). ICAM-1 was cleaved into the same sizes by PNGase F in control cells as well as asiatic acid-, corosolic acid-, maslinic acid- and ursolic acid-treated cells (Fig. 8). Moreover, ICAM-1 was digested to the same major band by Endo H in asiatic acid-, corosolic acid-, maslinic acid-, and ursolic acid-treated cells, while minor Endo H-resistant bands remained in asiatic acid- and maslinic acid-treated cells (Fig. 8). Since high-mannose-type and hybrid-type glycans are sensitive to Endo H digestion, these results indicate that asiatic acid, corosolic acid, and maslinic acid cause the accumulation of ICAM-1 linked to high-mannose-type and/or hybrid-type glycans.

Asiatic Acid Increased Co-localization of ICAM-1 with Calnexin, but Not GM130 We previously showed that ursolic acid inhibited intracellular trafficking from the ER to the Golgi apparatus and caused the accumulation of Endo H-sensitive ICAM-1 in the ER.13) Asiatic acid at 100 µM did not markedly affect the expression of cell-surface ICAM-1, but up-regulated the expression of the ICAM-1 protein by more than two-fold (Figs. 1B, 4A), implying that asiatic acid increased the amount of intracellular ICAM-1. In order to examine the subcellular localization of ICAM-1, A549 cells were preincubated with asiatic acid for 1 h and then incubated with IL-1α for 6 h. A549 cells were stained for ICAM-1, together with the cis-Golgi marker GM130 or ER marker calnexin. In control A549 cells, ICAM-1 was detected more abundantly at areas outside of calnexin and GM130, although some ICAM-1 co-localized with either GM130 or calnexin, indicating that ICAM-1 was mostly transported to the cell surface (Figs. 9A–D). In asiatic acid-treated A549 cells, the co-localization of ICAM-1 with calnexin was markedly increased, while that of ICAM-1 with GM130 was unaffected (Figs. 9A–D). It is im-
important to note that asiatic acid did not affect the morphology of the Golgi apparatus, but altered that of the ER to smaller or dot-like shapes (Figs. 9A, B). These results indicate that asiatic acid increases the amount of ICAM-1 in the ER.

**Asiatic Acid Delayed the Expression of Cell-Surface ICAM-1**

We performed time–course experiments on the expression of cell-surface ICAM-1 upon an IL-1α stimulation. In control A549 cells, cell-surface ICAM-1 steadily increased 1–3 h after the IL-1α stimulation and then gradually reached a plateau after 3–6 h (Fig. 10). Asiatic acid at 100 µM decreased the amount of cell-surface ICAM-1 during 2–4 h, but not 5–6 h (Fig. 10). These results indicate that asiatic acid delays the expression of cell-surface ICAM-1.

**Asiatic Acid, Corosolic Acid, and Maslinic Acid Inhibit α-Glucosidase, but Not α-Mannosidase**

We previously showed that ursolic acid inhibited yeast α-glucosidase, but not Jack bean α-mannosidase.13,14) Asiatic acid, corosolic acid, and maslinic acid inhibited yeast α-glucosidase activity in a dose-dependent manner (Figs. 11A–C). The IC₅₀ values of asiatic acid, corosolic acid, and maslinic acid were calculated to be 24.4±3.0, 8.5±2.5, and 19.0±3.1 µM (mean±standard error (S.E.) of three independent experiments), respectively. Unlike 1-deoxymannojirimycin, Jack bean α-mannosidase was not inhibited by asiatic acid, corosolic acid, maslinic acid, or ursolic acid at 100 µM (Fig. 11D).

**DISCUSSION**

Pentacyclic triterpenoids have been reported to exhibit various biological activities.7–12,15–18) As unique biological activities, we previously showed that ursolic acid inhibited the transport of ICAM-1 from the ER to the Golgi apparatus, and that betulinic acid and oleanolic acid did not inhibit the intracellular transport of ICAM-1, but affected its post-translational glycosylation most likely at the step of ER α-glucosidases.13,14) In the present study, we revealed that asiatic acid, corosolic acid, and maslinic acid interfered with the intracellular transport of ICAM-1 and caused the accumulation of ICAM-1 linked to Endo H-sensitive glycans, which are assumed to be high-mannose-type and/or hybrid-type glycans. Moreover, we showed that asiatic acid and maslinic acid up-regulated the expression of the ICAM-1 protein, whereas corosolic acid down-
regulated it at higher concentrations. Thus, these pentacyclic triterpenoids exert common and distinct effects on the protein expression and post-translational glycosylation of ICAM-1 and its intracellular transport to the cell surface.

A previous study reported that asiatic acid, corosolic acid, and maslinic acid inhibited $\alpha$-glucosidase from Saccharomyces cerevisiae at 30.03±0.41, 3.53±0.27, and 5.52±0.19 $\mu$g/mL, respectively,\(^{22}\) which were calculated to be 61, 7.5, and 12 $\mu$M, respectively. In another study, the IC\(_{50}\) values of asiatic acid and corosolic acid on yeast $\alpha$-glucosidase were found to be 100.2±0.2 and 17.2±0.9 $\mu$M, respectively.\(^{23}\) Consistent with these findings, we showed that asiatic acid, corosolic acid, and maslinic acid inhibited yeast $\alpha$-glucosidase, indicating that corosolic acid inhibits yeast $\alpha$-glucosidase more strongly than asiatic acid and maslinic acid. We previously demonstrated that betulinic acid and oleanolic acid inhibited $\alpha$-glucosidase.\(^{44}\) Betulinic acid and oleanolic acid affected N-linked glycan modifications to ICAM-1 most likely at the step of ER $\alpha$-glucosidases.\(^{44}\) However, in contrast to asiatic acid-, corosolic acid-, maslinic acid-, and ursolic acid-treated cells, ICAM-1 proteins were insensitive to Endo H digestion in betulinic acid- and oleanolic acid-treated cells.\(^{44}\) Thus, in addition to ER $\alpha$-glucosidases, asiatic acid, corosolic acid, maslinic acid, and ursolic acid appear to inhibit additional processes that cause the accumulation of the Endo H-sensitive glycans of ICAM-1.

Corosolic acid is known to exhibit various biological activities, including anti-inflammatory and anti-cancer activities.\(^{16}\) We showed that corosolic acid inhibited the expression of cell-surface ICAM-1 and decreased the molecular weight of ICAM-1. Moreover, it appeared that corosolic acid partially inhibited the mRNA expression of ICAM-1. In response to inflammatory cytokines, ICAM-1 is mainly up-regulated by NF-κB-dependent transcription.\(^{2}\) Previous studies reported that corosolic acid inhibited the NF-κB signaling pathway in several types of cells.\(^{24–27}\) Thus, the down-regulation of ICAM-1 protein expression by corosolic acid appears to be attributed partly to the inhibition of the ICAM-1 mRNA ex-
pression or the NF-κB signaling pathway. Corosolic acid is a close structural derivative of ursolic acid, which possesses an extra hydroxyl group at the C-2 position. We previously reported that ursolic acid inhibited the expression of cell-surface ICAM-1 without reducing its protein level at concentrations of 30–50 µM.13,14) ICAM-1 proteins in corosolic acid- and ursolic acid-treated cells were observed to be nearly the same size and equally sensitive to Endo H digestion. Therefore, corosolic acid and ursolic acid appear to share similar biological activities.

We found that corosolic acid reduced the number of adherent cells, but did not decrease MTT-reducing activity during the 7-h incubation in A549 cells. A previous study reported that corosolic acid induced apoptotic cell death in A549 cells in a 12-h and longer incubation.28) We speculate that the difference between the present results and previous finding may be attributed to the incubation time; however, culture conditions may also influence the sensitivity of A549 cells to corosolic acid.

Maslinic acid up-regulated the expression of the ICAM-1 protein, particularly at concentrations of 60 µM, whereas it augmented ICAM-1 mRNA expression at 60–100 µM.29–34) Furthermore, it decreased TNF-α-induced ICAM-1
In contrast to these findings, maslinic acid appeared to up-regulate the mRNA and protein expression of ICAM-1, at least in A549 cells. Further experiments are needed to elucidate the mechanisms by which maslinic acid augments the IL-1α-induced mRNA and protein expression of ICAM-1.

Asiatic acid up-regulated the expression of the ICAM-1 protein, particularly at concentrations of 100 µM. In contrast to maslinic acid, asiatic acid only slightly increased ICAM-1 mRNA expression or did not markedly affect the RelA nuclear translocation. Previous studies reported that asiatic acid inhibited the NF-κB signaling pathway in various types of cells. Asiatic acid was found to decrease TNF-α-induced ICAM-1 expression. Thus, asiatic acid appears to exert a slight effect on the IL-1α-induced ICAM-1 transcription, at least in A549 cells, and it may rather have affected translation or post-translational processes in order to up-regulate ICAM-1 protein levels. ICAM-1 possesses multiple N-linked glycosylation sites. In the ER, high-mannose-type sugar chains are transferred to newly-synthesized proteins and ER α-glucosidases I and II then sequentially remove glucose residues, which regulate the association with manectin, calnexin, and calreticulin. Castanospermine, as an ER α-glucosidase inhibitor, did not increase the amount of the ICAM-1 protein, suggesting that the inhibition of ER α-glucosidases does not cause an increase in the amount of the ICAM-1 protein. Further investigations are needed in order to elucidate the mechanisms by which asiatic acid and maslinic acid up-regulate the expression of the ICAM-1 protein.

A comparison between total ICAM-1 protein and cell-surface ICAM-1 indicated that asiatic acid, corosolic acid, and maslinic acid interfere with the intracellular trafficking of ICAM-1. In particular, asiatic acid at 100 µM increased the localization of the ICAM-1 protein in the ER and delayed the

![Graph](image-url)
Fig. 9. Asiatic Acid Augmented the Localization of ICAM-1 in the ER

(A–D) A549 cells were preincubated with or without asiatic acid for 1 h, and then incubated with or without IL-1α (0.25 ng/mL) for 6 h in the presence or absence of asiatic acid (100 μM) as the final concentrations. Cells were stained for ICAM-1, together with the cis-Golgi marker GM130 (A and C) or ER marker calnexin (B and D). Data are representative of three independent experiments (A and B). The co-localization of ICAM-1 with GM130 or calnexin is shown as the mean±S.E. of three independent experiments (C and D). The Student’s t-test was used to evaluate the significance of differences. *** p<0.001, ns: not significant.
expression of cell-surface ICAM-1. We previously reported that ursolic acid at 50 µM induced the fragmentation of the Golgi apparatus,\textsuperscript{13} which appears to account for the inhibition of glycoprotein transport from the ER to the Golgi apparatus. In asiatic acid-treated cells, the ER was changed to smaller or dot-like structures, while the Golgi apparatus remained unaltered. This may explain why ICAM-1 remains longer in the ER, but is ultimately transported to the cell surface in asiatic acid-treated cells. We previously reported that tunicamycin caused an unglycosylated form of ICAM-1 and inhibited its expression to the cell surface.\textsuperscript{13} Castanospermine (an inhibitor of ER α-glucosidases I and II), 1-deoxymannojirimycin (an inhibitor of Golgi α-mannosidase I), and swainsonine (an inhibitor of Golgi α-mannosidase II) did not inhibit the expression of cell surface ICAM-1, but decreased its molecular weight.\textsuperscript{13,14} Thus, the inhibition of α-glucosidases or α-mannosidases may be dispensable for the cell surface expression of the ICAM-1 protein.

In conclusion, we herein revealed that asiatic acid, corosolic acid, and maslinic acid interfered with the intracellular trafficking of ICAM-1 to the cell surface. Moreover, asiatic acid and maslinic acid increased the amount of the ICAM-1 protein, while corosolic acid inhibited the expression of the ICAM-1 protein. Cell-surface adhesion molecules, such as ICAM-1, play an essential role in inflammatory responses and cancer metastasis.\textsuperscript{4–6} Glycosylation regulates the function of endothelial adhesion molecules, such ICAM-1.\textsuperscript{45} Hence, the inhibition of cell-surface adhesion molecules is considered to serve as anti-inflammatory and anti-cancer agents. Pentacyclic triterpenoids are known to be major components in many plants and are composed of a large number of structural derivatives.\textsuperscript{7,8} Pentacyclic triterpenoids are assumed to target multiple cellular proteins and exhibit diverse biological activi-
ties. Further structure–activity relationships and the identification of target proteins may be important for the development of more effective anti-inflammatory and anti-cancer agents.

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Conflict of Interest The authors declare no conflict of interest.

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