

Selinidin Suppresses IgE-Mediated Mast Cell Activation by Inhibiting Multiple Steps of FcεRI Signaling

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IgE-mediated mast cell activation is critical for development of allergic inflammation. We have recently found that selinidin, one of the coumarin derivatives isolated from *Angelica keiskei*, attenuates mast cell degranulation following engagement of the high-affinity receptor for IgE (FcεRI) with IgE and antigen. In the present study, we investigated the effects of selinidin on intracellular signaling and mast cell activation employing bone marrow-derived mast cells. Here, we report that selinidin attenuates the release of β-hexosaminidase, synthesis of leukotriene C₄, and production of tumor necrosis factor-α without affecting IgE-FcεRI binding. Furthermore, biochemical analyses of the FcεRI-mediated signaling pathway demonstrated that selinidin decreases phosphorylation of phospholipase C-γ1, p38 mitogen-activated protein kinase, and IκB-α upon FcεRI stimulation. These results suggest that this compound suppresses IgE-mediated mast cell activation by inhibiting multiple steps of FcεRI-dependent signaling pathways and would be beneficial for the prevention of allergic inflammation.

Key words IgE; mast cell; high-affinity receptor for IgE (FcεRI); selinidin; degranulation; cytokine production

Mast cells express the high-affinity receptor for IgE (FcεRI) on the cell surface, which plays crucial roles in the development of allergic inflammation. FcεRI is mainly expressed on mast cells and basophils as a tetramer comprised of the IgE-binding α-chain, two types of signaling subunit, a β-chain, and a disulfide-linked homodimer of γ-chains.¹⁾ IgE-bound FcεRI is engaged by multivalent antigens, and a series of FcεRI signaling is evoked. Activation of signaling is critical for a variety of mast cell functions, such as release of preformed intragranular chemical mediators, new synthesis of eicosanoids, and production of proinflammatory cytokines.²⁾ Upon FcεRI engagement, the src-family tyrosine kinase Lyn immediately phosphorylates the β-chain, which in turn leads to subsequent tyrosine phosphorylation of the γ-chain, Syk, and phospholipase C (PLC)-γ, activation of protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) family members.³⁾ In addition, an alternative signaling pathway by which the src-family tyrosine kinase Fyn initiates the phosphoinositide 3-kinase (PI3K) cascade is activated.⁴⁾ Previous studies reported that suppression of these signaling molecules has substantial inhibitory effects on the release of histamine and generation of arachidonic acid metabolites in mast cells.^{5–7)}

Angelica keiskei (known as ashitaba in Japan, Umbelliferae) is a hardy perennial plant, growing mainly in warm areas along the Pacific coast of Japan. *A. keiskei* is edible and available as a vegetable. The yellow juice from the cut end of the stem of *A. keiskei* contains abundant chalcones, coumarins, and flavanones.⁸⁾ It was reported that these natural products have various beneficial effects including antitumor and antibacterial activity.^{8–11)} We have recently identified selinidin isolated from the extract of *A. keiskei* as an antiallergic compound in screening employing rat basophilic leukemia (RBL)-2H3 cells. Since selinidin (Fig. 1), one of the coumarin derivatives, had an inhibitory effect on degranulation of RBL-2H3 cells upon engagement of FcεRI with IgE and antigen, we investigated the effects of selinidin on IgE-mediated intracellular signaling and mast

cell activation in more detail employing primary mouse bone marrow-derived mast cells (BMMCs).

MATERIALS AND METHODS

Mice C57BL/6J mice were purchased from Charles River Laboratories Japan (Kanagawa, Japan). FcεRI β-chain-deficient mice were bred in the animal faculty under specific pathogen-free conditions at Nihon University School of Medicine. All experiments were performed in accordance with the guidelines for the care and use of laboratory animals of Nihon University.

Isolation of Selinidin Purification of selinidin was performed as previously described.⁸⁾ Briefly, the powdered yellow exudates obtained from the stems of *A. keiskei* were fractionated with *n*-hexane, ethyl acetate, *n*-butanol, and H₂O. Selinidin was isolated from the ethyl acetate-soluble fraction and its structure was confirmed with NMR. Selinidin was dissolved in dimethyl sulfoxide (DMSO) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and stored at –20 °C. The chemical structure of selinidin is shown in Fig. 1.

Reagents and Antibodies Anti-FcεRI β-chain monoclonal antibody (mAb) (clone: JRK), which was a kind gift from Dr. Juan Rivera of the US National Institutes of Health (NIH), was prepared from ascitic fluid in our laboratory. Anti-trinitrophenyl (TNP)-mouse IgE (clone: IgE-3) was purchased from BD PharMingen (San Diego, CA, U.S.A.) and labeled with Alexa Fluor488 dye (Invitrogen/Molecular

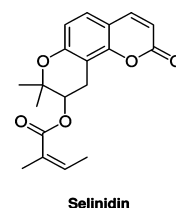


Fig. 1. Chemical Structure of Selinidin

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Probes, Eugene, OR, U.S.A.). TNP-bovine serum albumin (BSA) was purchased from LSL (Tokyo, Japan), anti-phosphotyrosine mAb conjugated with horseradish peroxidase (HRP) (clone: PY20) from GE Healthcare UK (Buckinghamshire, U.K.), and anti-Fc ϵ RI γ -chain polyclonal antibody from Upstate (Lake Placid, NY, U.S.A.). Polyclonal antibodies to phospho-PLC γ 1 (Tyr783), PLC γ 1, phospho-ERK1/2 (Thr202/Tyr204), ERK1/2, phospho-JNK1/2 (Thr183/Tyr185), JNK1/2, phospho-p38 MAPK (Thr180/Tyr182), p38 MAPK, phospho-I κ B- α (Ser32), and I κ B- α were purchased from Cell Signaling Technology (Danvers, MA, U.S.A.). Armenian hamster anti-mouse Fc ϵ RI α -chain mAb (clone: MAR-1) and Armenian hamster IgG were purchased from eBioscience (San Diego, CA, U.S.A.). Anti-Lyn polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

Cell Culture BMMCs were prepared as previously described.¹²⁾ Briefly, bone marrow cells from femurs of 4-week-old female C57BL/6J mice or Fc ϵ RI β -chain-deficient mice were cultured for 4–8 weeks in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, U.S.A.) supplemented with 10% (v/v) of heat-inactivated fetal bovine serum (FBS) (SAFC Biosciences, Lenexa, KS, U.S.A.), 4 ng/ml of recombinant murine interleukin (IL)-3 (PeproTech, London, U.K.), 71.6 μ M of 2-mercaptoethanol, 100 μ M of MEM Non-Essential Amino Acids (Gibco/Invitrogen), 1 mM of sodium pyruvate, 100 U/ml of benzyl penicillin potassium, and 100 μ g/ml of streptomycin sulfate. Before all experiments, cell surface Fc ϵ RI expression was confirmed using flow cytometric analyses.

Cell Stimulation BMMCs (5×10^6 /ml) were sensitized with 1 μ g/ml of anti-TNP-IgE for 2 h at 37 °C and then washed twice with 1 $\times$ Tyrode's buffer (NaCl 126 mM, KCl 4 mM, KH₂PO₄ 641 μ M, HEPES 10 mM, D-glucose 5.6 mM, CaCl₂ 1 mM, MgCl₂ 600 μ M, and 0.1% BSA), or with RPMI-1640 medium supplemented with 2% (v/v) FBS. The IgE-sensitized BMMCs were treated with or without selinidin for 30 min at 37 °C. The cells were stimulated with the indicated concentrations of TNP-BSA.

Flow Cytometric Analyses For detecting apoptotic cells, selinidin-treated cells (1×10^5) were stained with annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) for 15 min at room temperature and then analyzed employing FACSCalibur (Becton Dickinson Biosciences, San Jose, CA, U.S.A.). Double-negative cells [annexin V-FITC (–)/PI (–)] were estimated as viable cells. We analyzed the effects of selinidin on IgE-Fc ϵ RI binding as follows: 1) primary BMMCs (2×10^5) were incubated with vehicle or selinidin (50 μ g/ml) for 30 min at 37 °C, and the cells were washed with Tris-buffered-saline (TBS, pH 7.6) and then stained with 0.5 μ g of IgE-Alexa488; or 2) 0.5 μ g of IgE-Alexa488 was incubated with vehicle or selinidin (50 μ g/ml) for 30 min at 37 °C, and primary BMMCs (2×10^5) were stained with the selinidin-treated or untreated IgE-Alexa488. Fc ϵ RI expression on the cell surface was analyzed after Fc ϵ RI α -chain staining with FITC-labeled anti-mouse Fc ϵ RI α -chain mAb. BMMCs derived from Fc ϵ RI β -chain-deficient mice were used as Fc ϵ RI-negative cells. Mean fluorescence intensities of these cells were evaluated.

Assay of Degranulation Degranulation was determined based on β -hexosaminidase release as previously described.¹²⁾

Briefly, IgE-sensitized BMMCs (2×10^5) were stimulated with 3 ng/ml of TNP-BSA for 30 min in 0.2 ml of 1 $\times$ Tyrode's buffer. For preparation of the total amount of β -hexosaminidase, cells were lysed with 0.5% Triton X-100. The supernatant or the total lysates were incubated with *p*-nitrophenyl-2-acetamido-2-deoxy- β -D-glucopyranoside 3.8 μ M for 40 min at 37 °C. The reaction was stopped by the addition of glycine 0.2 M (pH 11.0), and then the absorbance was measured at 450 nm. The percentage of β -hexosaminidase release was calculated as follows: (the absorbance of the supernatant of stimulated or unstimulated cells)/(the absorbance of the total lysates) $\times$ 100.

Measurement of leukotriene (LT) C_4 IgE-sensitized BMMCs (2×10^5) were stimulated with TNP-BSA 3 ng/ml for 30 min in 0.2 ml of 1 $\times$ Tyrode's buffer. The supernatant was analyzed with an LTC₄ EIA kit (Cayman Chemical, Ann Arbor, MI, U.S.A.).

Measurement of TNF- α and IL-6 IgE-sensitized BMMCs (2×10^5) were stimulated with TNP-BSA 10 ng/ml for 8 h in 0.2 ml of RPMI-1640 medium supplemented with 2% (v/v) FBS. TNF- α and IL-6 production were analyzed with specific ELISA kits (BioSource International, Camarillo, CA, U.S.A.).

Immunoblotting The antigen-stimulated cells (5×10^5) were lysed with sample buffer [Tris-HCl 64 mM (pH 6.8), 10% glycerol, 2% SDS, 2-mercaptoethanol 734 mM] and boiled for 15 min at 100 °C. The cell lysates were centrifuged for 5 min at 20400 $\times g$, and the supernatant was collected. For I κ B- α blotting, the stimulated cells (2×10^6) were lysed with lysis buffer [TBS containing 1% Nonidet P-40, phenylmethylsulfonyl fluoride (PMSF) 2 mM, aprotinin 10 μ g/ml, sodium orthovanadate 1 mM, pepstatin A 2 μ g/ml, leupeptin 2 μ g/ml, NaF 50 mM] for 15 min on ice. The cell lysates were centrifuged for 15 min at 17800 $\times g$. The supernatant was mixed with an equal volume of the sample buffer and boiled for 5 min at 100 °C. For immunoprecipitation, cells (1×10^7) were lysed with lysis buffer for 15 min on ice. The cell lysates were centrifuged for 15 min at 17800 $\times g$. Specific antibody (10 μ g) was added to the supernatant and incubated for 3 h at 4 °C. The immunocomplex was precipitated with 10 μ g of protein-A-sepharose and then boiled for 5 min at 100 °C.

Whole-cell lysates and immunoprecipitates were separated on 10% or 10–20% gradient SDS-PAGE and transferred to a PVDF membrane. The membrane was incubated with appropriate primary and secondary antibodies. Signals were detected with chemiluminescence using an ECL detection system (GE Healthcare). The signal intensities were quantified with NIH Image software and the increases were calculated by the relative amount of phospho protein to each non-phospho loading control.

Statistical Analysis Statistical analysis was performed with Student's *t*-test. Probability values of less than 0.05 were considered to represent statistically significant differences.

RESULTS

Toxic Effects of Selinidin on BMMCs First, we examined the effects of selinidin on mast cell viability. Primary BMMCs were incubated with selinidin for 30 min and further cultured for 0.5 or 8 h in the absence of selinidin. Viable cells

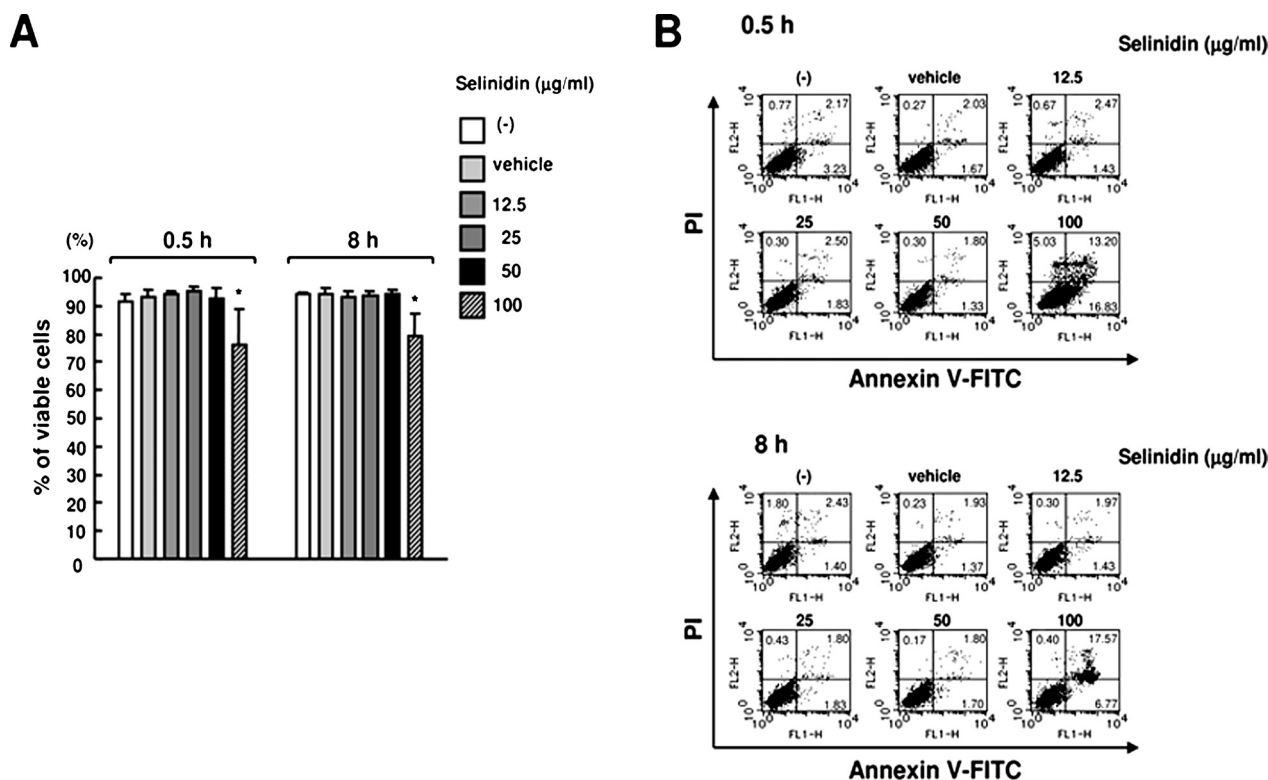


Fig. 2. Mast Cell Viability Is Lowered by Treatment with High Concentrations of Selinidin

(A) BMMCs (1×10^6 /ml) were pretreated with selinidin at the indicated concentrations for 30 min and further incubated with selinidin-free medium for 0.5 or 8 h. The cells (1×10^5) were stained with annexin V-FITC and PI. Double-negative annexin V-FITC and PI cells are shown as viable cells. Data shown are the mean $\pm$ S.D. of four independent experiments. (B) Data shown are one representative of four independent experiments with similar results. In A, Student's *t*-test was performed between vehicle-treated cells and selinidin-treated cells, * $p < 0.05$.

were evaluated by double staining with annexin V-FITC and PI. As shown in Figs. 2A and B, selinidin had no cytotoxic effects on BMMCs in range of 12.5–50 μ g/ml, whereas a high concentration of selinidin (100 μ g/ml) showed cytotoxic effects at both time periods.

Effects of Selinidin on Degranulation, LTC₄ Synthesis, and Cytokine Production in BMMCs We first examined the effects of selinidin on degranulation after Fc ϵ RI stimulation. As shown in Fig. 3A, 50 μ g/ml of selinidin attenuated the release of the β -hexosaminidase (a representative intragranular enzyme) in BMMCs stimulated with IgE and antigen. Since new synthesis of eicosanoids as well as release of intragranular enzymes is an immediate response of antigen-stimulated mast cells,¹³⁾ next we examined the effects of selinidin on the production of LTC₄. Figure 3B shows that selinidin apparently suppressed LTC₄ synthesis upon Fc ϵ RI engagement at the same concentration as in Fig. 3A.

To elucidate the effects of selinidin on the secretion of proinflammatory cytokines, we examined TNF- α and IL-6 production at protein levels using each specific ELISA. TNF- α production as well as β -hexosaminidase release was suppressed by culture with 50 μ g/ml of selinidin, whereas IL-6 production was not attenuated at the same concentration (Figs. 3C, D). When BMMCs were pretreated with 12.5 or 25 μ g/ml of selinidin, Fc ϵ RI-induced IL-6 production was slightly increased as compared with that of selinidin-untreated cells. However, selinidin itself failed to induce IL-6 production by BMMCs (data not shown). Collectively, these results suggest antiallergic activity of selinidin, which inhibits Fc ϵ RI-mediated mediator release and TNF- α produc-

tion in mast cells.

Selinidin Does Not Interfere with IgE-Fc ϵ RI Binding and Fc ϵ RI Expression Binding of IgE to Fc ϵ RI is one of the essential processes to initiate mast cell activation, and we therefore examined whether selinidin reduces the binding between IgE and Fc ϵ RI. As shown in Fig. 4A, IgE-Alexa488 did not bind to Fc ϵ RI-negative BMMCs derived from β -chain-deficient mice, indicating high-affinity binding between IgE and Fc ϵ RI on BMMCs. Primary BMMCs and IgE-Alexa488 were pretreated with vehicle or 50 μ g/ml of selinidin for 30 min, respectively. Figure 4B shows that pretreatment of BMMCs with selinidin had no inhibitory effects on the binding of IgE-Alexa488 to BMMCs. Pretreatment of IgE-Alexa488 with selinidin also did not have any inhibitory effects on its binding to Fc ϵ RI (Fig. 4C). Moreover, selinidin did not alter Fc ϵ RI expression on the cell surface (Fig. 4D). Consistent with this, the amount of Fc ϵ RI α -chain protein was not altered in selinidin-treated BMMCs (Fig. 4E). From these results, it was concluded that selinidin has no effects on IgE-Fc ϵ RI binding and cell surface Fc ϵ RI expression.

Selinidin Inhibits Tyrosine Phosphorylation of Fc ϵ RI β -chain and PLC γ 1 in BMMCs upon Fc ϵ RI Stimulation The initial signals *via* Fc ϵ RI are mediated through tyrosyl transphosphorylation,¹⁴⁾ and we therefore examined the effects of selinidin on total tyrosine phosphorylation in BMMCs stimulated with IgE and antigen. As shown in Fig. 5A, selinidin had inhibitory effects on tyrosine phosphorylation of several protein species (approximately 110, 94, 34, and 29 kDa, indicated with asterisks). These results suggest

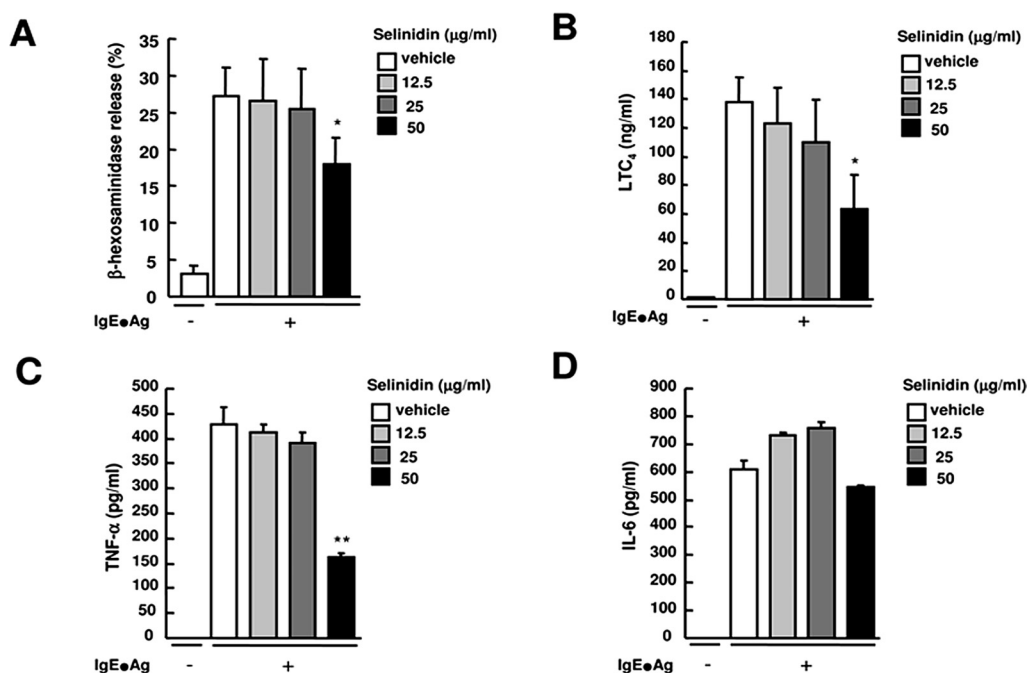


Fig. 3. Effects of Selinidin on Degranulation, LTC₄ Production, and Cytokine Production upon FcεRI Stimulation

(A) Degranulation was evaluated based on β -hexosaminidase release. IgE-sensitized BMMCs (1×10^6 /ml) were treated with selinidin for 30 min at the indicated concentrations. The cells (2×10^5) were stimulated with 3 ng/ml of antigen for 30 min in 0.2 ml of 1×Tyrode's buffer. (B) LTC₄ production was measured in specific EIA. Selinidin-treated BMMCs (2×10^5) were stimulated with antigen as in A. Cytokine production was analyzed with specific ELISA for TNF- α (C) or IL-6 (D). IgE-sensitized BMMCs (1×10^6 /ml) were treated with selinidin as in A. The cells (2×10^5) were stimulated with 10 ng/ml of antigen for 8 h in 0.2 ml of RPMI-1640 medium containing 2% (v/v) FBS. In A–D, data shown are the mean $\pm$ S.D. of four independent experiments, and Student's *t*-test was performed between vehicle-treated cells and selinidin-treated cells, **p* < 0.05; ***p* < 0.01.

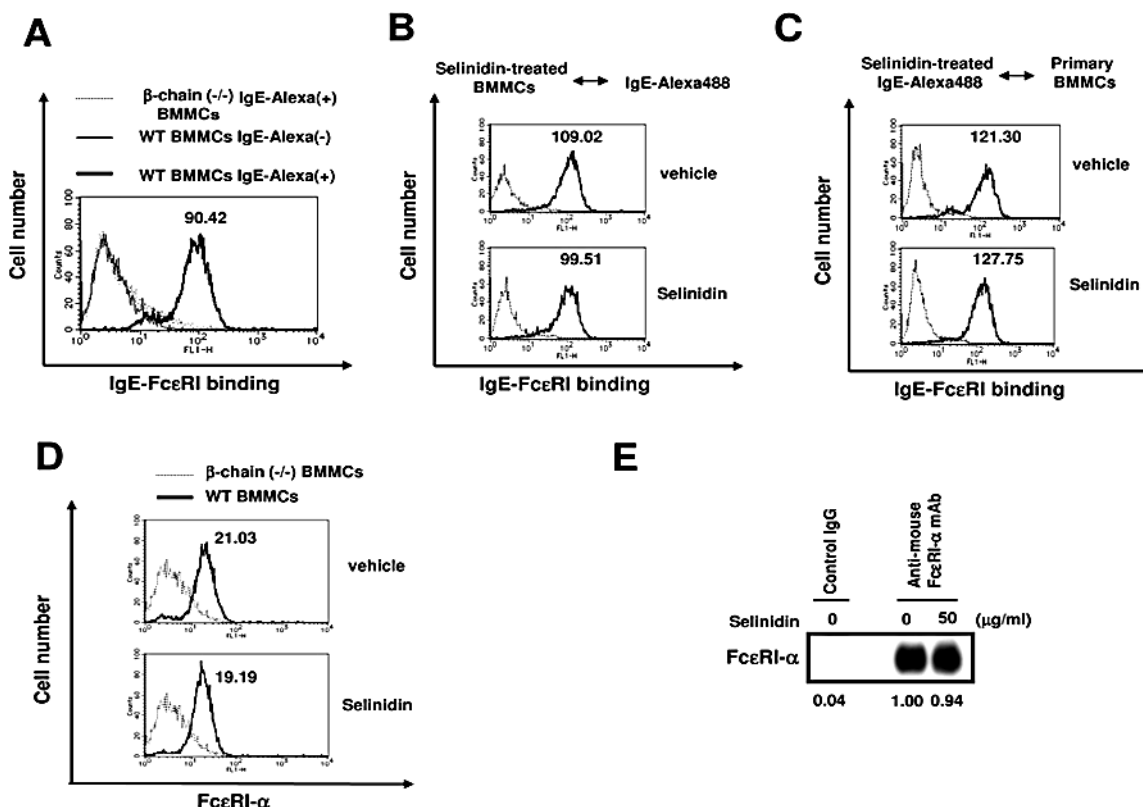


Fig. 4. Selinidin Did Not Affect IgE-FcεRI Binding and Cell Surface FcεRI Expression

(A) FcεRI β -chain (-/-) BMMCs (2×10^5) and wild-type BMMCs (2×10^5) were stained with IgE-Alexa488 (0.5 μ g). (B) BMMCs (1×10^6 /ml) were treated with or without 50 μ g/ml of selinidin for 30 min. The cells (2×10^5) were stained with IgE-Alexa488 (0.5 μ g). (C) IgE-Alexa488 (0.5 μ g) was treated with or without 50 μ g/ml of selinidin for 30 min. BMMCs (2×10^5) were stained with the selinidin-treated IgE-Alexa488 or the untreated IgE-Alexa488. The cells bound to IgE-Alexa488 were analyzed employing flow cytometry. (D) BMMCs were treated with selinidin as in A. The cells (2×10^5) were stained with FITC-labeled anti-FcεRI α -chain mAb. In D, β -chain (-/-) BMMCs were used as FcεRI-negative cells. (E) BMMCs (1×10^7 /ml) were treated with or without 50 μ g/ml of selinidin for 30 min. The cells were lysed with lysis buffer. Whole-cell lysates were incubated with anti-mouse FcεRI α -chain mAb or Armenian hamster IgG. The immunoprecipitates were analyzed by immunoblotting with anti-FcεRI α -chain mAb. Similar results were obtained from three independent experiments.

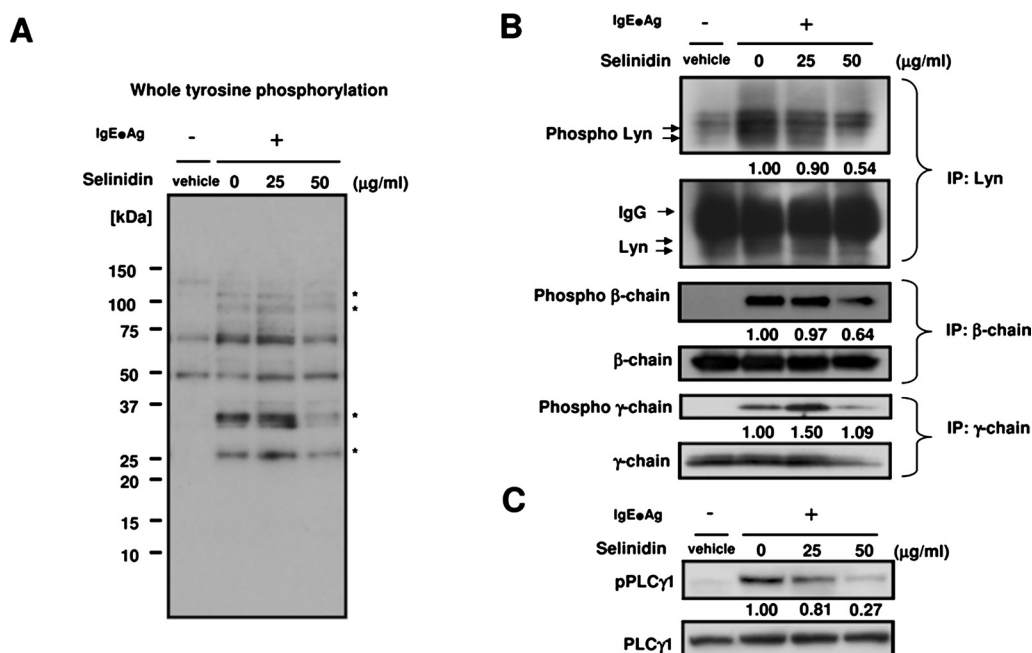


Fig. 5. Effects of Selinidin on Mast Cell Signal Transduction

IgE-sensitized BMMCs (1×10^6 /ml) were treated with selinidin for 30 min at the indicated concentrations. The cells were stimulated with 3 ng/ml of antigen for 3 min. The stimulated cells were lysed with sample buffer. Total tyrosine phosphorylation was detected by immunoblotting with anti-phosphotyrosine mAb (PY20) (A). The IgE-sensitized cells (1×10^6 /ml) were treated with selinidin and then stimulated with 30 ng/ml of antigen for 3 min. The stimulated cells were lysed with the lysis buffer. Whole-cell lysates were incubated with anti-Lyn, anti-β-chain, or anti-γ-chain antibody for 3 h at 4°C. The immunoprecipitates were analyzed by immunoblotting with anti-phosphotyrosine mAb (PY20). The same membrane was reprobed with anti-Lyn, anti-β-chain, or anti-γ-chain antibody, respectively (B). Whole-cell lysates were prepared as in A and analyzed by immunoblotting with anti-phospho-PLCγ1 antibody (C). In B and C, the increases were calculated based on the relative amount of phospho proteins to nonphospho proteins. Results shown are one representative of three independent experiments with similar results.

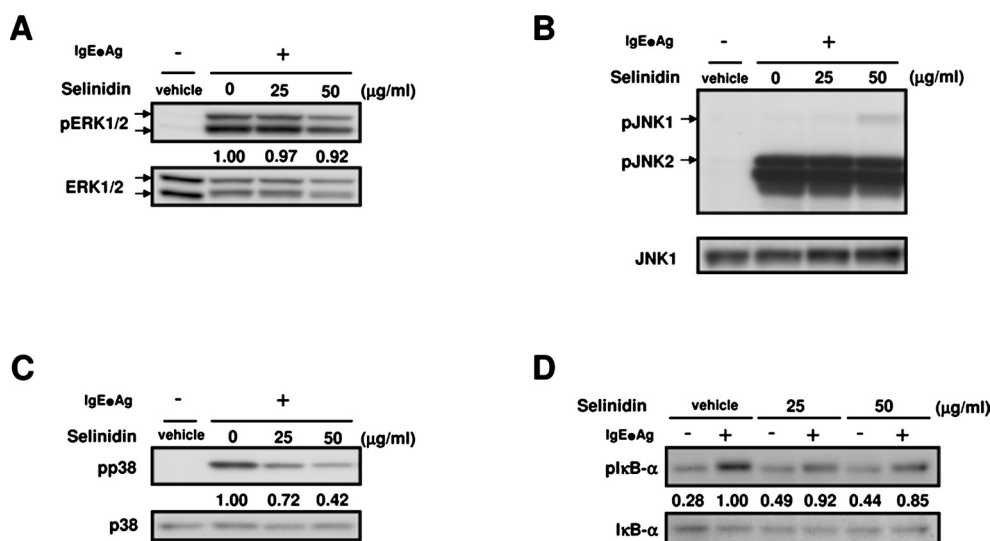


Fig. 6. Selinidin Inhibits Phosphorylation of p38 MAPK and IκB-α Upon FcεRI Engagement

IgE-sensitized BMMCs (1×10^6 /ml) were treated with selinidin for 30 min at the indicated concentrations. The cells (0.5 – 2×10^6 /ml) were stimulated with 3 ng/ml of antigen for 3 min. The stimulated cells were lysed with lysis buffer or sample buffer. Signals were detected by immunoblotting with specific antibodies to phospho-ERK1/2 (A), phospho-JNK1/2 (B), phospho-p38 MAPK (C), or phospho-IκB-α (D), followed by reprobing the same blot with anti-ERK1/2, anti-JNK1/2, anti-p38 MAPK, or anti-IκB-α antibody, respectively. In A–D, the fold increases were calculated based on the relative amount of phospho proteins to nonphospho proteins. Results shown are one representative of three independent experiments with similar results.

that this compound is likely to affect the activation of proximal signaling molecules upon FcεRI engagement. As expected, tyrosine phosphorylation of Lyn and the FcεRI β-chain was apparently suppressed by treatment with selinidin 50 μg/ml (Fig. 5B). Unlike tyrosine phosphorylation of Lyn and the FcεRI β-chain, however, the phosphorylation of the FcεRI γ-chain was not altered at the same concentration. Interestingly, selinidin considerably inhibited tyrosine

phosphorylation of PLCγ1, which is a downstream molecule of signaling *via* the FcεRI γ-chain (Fig. 5C). It is therefore likely that selinidin directly inhibits the PLCγ1 phosphorylation elicited by FcεRI stimulation.

Selinidin Inhibits Phosphorylation of p38 MAPK and IκB-α in BMMCs upon FcεRI Stimulation MAPK family members including ERK1/2, JNK1/2, and p38 MAPK play critical roles in proinflammatory cytokine production in

mast cells,^{15,16)} and we thus examined whether selinidin inhibits phosphorylation of MAPK family members in BMMCs following Fc ϵ RI cross-linking. As shown in Fig. 6A and B, selinidin did not inhibit ERK1/2 and JNK2 phosphorylation. We could not detect apparent JNK1 phosphorylation induced by antigen stimulation. When BMMCs were pretreated with 50 μ g/ml of selinidin, the phosphorylated form of JNK1 appeared upon Fc ϵ RI stimulation. On the contrary, p38 MAPK phosphorylation was clearly suppressed by treatment with selinidin in a concentration-dependent manner (Fig. 6C). Moreover, Ser32 phosphorylation of I κ B- α , known as a regulator of NF- κ B translocation, was somewhat decreased in the selinidin-treated BMMCs upon Fc ϵ RI engagement.

DISCUSSION

In the present study, we found that selinidin, a coumarin derived from *A. keiskei*, attenuates degranulation, LTC₄ synthesis, and TNF- α production in antigen-stimulated BMMCs. Furthermore, we showed that selinidin modulates Fc ϵ RI-mediated signaling although it does not prevent IgE-Fc ϵ RI binding and cell surface Fc ϵ RI expression. These results suggest that selinidin suppresses IgE-mediated mast cell activation by inhibiting signal transduction pathways through Fc ϵ RI.

As demonstrated in Fig. 5C, selinidin significantly inhibited the tyrosine phosphorylation of PLC γ 1 induced by antigen stimulation. PLC γ 1 activates generation of inositol-3 phosphate, which triggers subsequent activation of calcium signals.³⁾ Such PLC γ -dependent calcium responses are essential for both mast cell degranulation and LTC₄ production,¹⁷⁾ we therefore consider that the selinidin probably reduces β -hexosaminidase release and LTC₄ synthesis through inhibition of PLC γ 1 activation. Interestingly, selinidin efficiently inhibits platelet aggregation *via* the collagen receptor glycoprotein VI that physically associates with the Fc ϵ RI γ -chain.¹⁸⁾ Previous studies might support part of the suppressive effects of selinidin on PLC γ 1 phosphorylation demonstrated in our results in the present study, because the platelet aggregation is largely dependent on PLC γ -mediated intracellular signaling.¹⁹⁾

Optimal LTC₄ synthesis requires not only calcium signaling but also MAPK signaling.²⁰⁾ ERK1/2 and p38 MAPK regulate cellular localization of 5-lipoxygenase (5-LO) to facilitate leukotriene synthesis.²¹⁾ Since selinidin inhibited phosphorylation of p38 MAPK, but not ERK1/2 in a concentration-dependent manner (Fig. 6C), we believe that the decreased p38 MAPK phosphorylation also results in the reduction of LTC₄ synthesis.

It was reported that MAPK family members regulate expression of proinflammatory cytokines such as TNF- α and IL-6 in a NF- κ B-dependent fashion.^{22,23)} Both TNF- α and IL-6 genes have several binding sites to the NF- κ B in their 5' untranslated region (UTR). Selinidin somewhat inhibited Ser32 phosphorylation of I κ B- α (Fig. 6D) that initiates NF- κ B translocation into the nucleus.²⁴⁾ However, our experiments using ELISA showed that selinidin has different effects on TNF- α and IL-6 production (Figs. 3C, D). This discrepancy can be explained by differences in posttranscriptional control between TNF- α and IL-6. In antigen-stimu-

lated mast cells, the stability of TNF- α mRNA is much lower than that of IL-6 mRNA,²⁵⁾ although both mRNAs possess AUUUA-rich elements in their 3'UTR.²⁶⁾ p38 MAPK increases the stability of TNF- α mRNA rather than that of IL-6 mRNA through activation of MAPK-activated protein kinase2.²⁷⁾ Based on these findings, we currently speculate that the inhibition of p38 MAPK activation by selinidin may result in preferential reduction of TNF- α expression. In addition to the above speculation, we believe that the suppression of degranulation by selinidin may also be involved in the difference in cytokine production, because mast cells spontaneously store TNF- α but not IL-6 proteins in the granule.²⁸⁾

In conclusion, our findings in the present study showed potential for selinidin as a natural antiallergic agent. *A. keiskei* containing selinidin may be a useful food for prevention of allergic inflammation and disorders in daily life. However, high concentrations of selinidin decreased mast cell viability, suggesting that excessive intake of selinidin could cause side effects in humans.

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