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Suppression of phospholipase C γ 1 phosphorylation by cinnamaldehyde inhibits antigen-induced extracellular calcium influx and degranulation in mucosal mast cells

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ABSTRACT

Antigen-IgE-mediated mucosal mast-cell activation is critical in the development of food allergies. Cinnamaldehyde, a major constituent of *Cinnamomi cortex*, dose-dependently inhibited the antigen-IgE-induced degranulation of mucosal-type bone-marrow derived mast cells (mBMMCs) and RBL-2H3 cells. Cinnamaldehyde also suppressed the elevation of the intracellular Ca²⁺ level that is induced by the extracellular Ca²⁺ influx in antigen-IgE-stimulated mBMMCs. Furthermore, tyrosine phosphorylation of phospholipase C (PLC) γ 1, which is a crucial activation switch for the intracellular Ca²⁺ mobilization in mast cells, was attenuated by cinnamaldehyde. Together, our results demonstrated that cinnamaldehyde suppressed the intracellular Ca²⁺ mobilization and the degranulation of mucosal mast cells by inhibiting the activity of the IgE receptor-PLC γ -Ca²⁺ influx pathway. These findings suggest that cinnamaldehyde may have therapeutic potential in mucosal mast cell-related allergic diseases, such as food allergies.

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1. Introduction

Mast cells play a pivotal role in the initiation of the inflammatory reactions that are associated with allergic disorders. The aggregation of high-affinity IgE receptors (Fc ϵ RI) on mast cells triggers their degranulation, resulting in a release of inflammatory mediators, such as proteases, inflammatory cytokines and lipid mediators, which are extensively involved in the pathogenesis of allergic diseases [1]. There are two distinct populations of mast cells, namely, mucosal mast cells and connective-tissue mast cells. Mucosal mast cells play a more important role in gastrointestinal anaphylaxis and protective responses against parasitic infections than connective-tissue mast cells [2]. Previously, we established a mouse model of food allergy and demonstrated that the mucosal mast cells play an essential role in the development of food allergies [3].

At present, no therapeutic drugs for food allergies have been developed. Connective tissue mast cell stabilizers (tranilast, ketotifen and cromolyn) are frequently used for the treatment of various allergic disorders. However, these agents have no effect on mucosal mast cells in vitro [4] and failed to improve allergic diarrhea in our food allergy mouse model [3]. Consequently, novel approaches are being explored to develop innovative therapies. Several herbal drugs in Japanese traditional medicine are known as anti-allergic agents. Recently, we demonstrated that Kakkonto, a traditional Japanese herbal medicine that originated in China, significantly suppressed the occurrence of allergic diarrhea in our food allergy mouse model [3] and also inhibited the degranulation of mucosal mast cells in vitro [4]. Kakkonto is composed of seven medicinal herbs, including *Cinnamomi cortex*. Simultaneously, to explore herbal medicines that have an inhibitory effect on the activation of mucosal mast cells, we used RBL-2H3 cells, a tumor analog of mucosal mast cells, to screen hot-water extracts from 80 commonly used Japanese herbal medicines (provided by Joint Usage/Research Center for Science-Based Natural Medicine, Institute of Natural Medicine, University of Toyama and the Knowledge Cluster Initiative Program [Second Stage] from the Ministry of Education, Culture, Sports, Science and Technology of Japan). Among them, we found that 5 medicinal herbs, including *Cinnamomi cortex*, significantly suppressed the degranulation of RBL-2H3 cells.

Abbreviations: DNP, dinitrophenyl; Fc ϵ RI, high affinity IgE receptors; IgE, immunoglobulin E; mBMMCs, mucosal-type bone-marrow derived mast cells; PLC γ 1, phospholipase C γ 1.

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Cinnamomi cortex, one of the major medicinal herbs of traditional Japanese and Chinese herbal medicines, has been widely used for the treatment of headache, fever, hot flashes, cold and physical pain. *Cinnamomi cortex* has been shown to have anti-inflammatory activity in some experimental models of acute inflammation [5]. In the present study, we investigated the direct effects of *Cinnamomi cortex* and cinnamaldehyde, a major constituent of *Cinnamomi cortex*, on the antigen-evoked activation of RBL-2H3 cells and mucosal-type murine bone-marrow derived mast cells (mBMMCs).

2. Materials and methods

2.1. Reagents and antibodies

A water extract of *Cinnamomi cortex* purchased from Tsumura Co. (Tokyo, Japan) was dissolved in water at 10 mg/ml. Cinnamaldehyde, salicyl aldehyde, cinnamic acid, and epicatechin (Nacalai Tesque, Kyoto, Japan) were dissolved in DMSO at 1 M and diluted in loading buffer to produce the stock solutions. Mouse monoclonal anti-dinitrophenyl (DNP) IgE was purchased from Yamasa Corporation (Tokyo, Japan). A23187 was purchased from Sigma (St. Louis, MO). Anti-Lyn antibody (sc-15) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-specific Lyn (#2731), anti-PLC γ 1 (#2822), and anti-phospho-specific PLC γ 1 (#2821) antibodies were obtained from Cell Signaling Technology Inc. (Danvers, MA).

2.2. Cell culture

RBL-2H3 cells (kindly provided by Dr. Hidetaka Yakura, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan) were used for the immunoprecipitation and western blotting because large numbers of cells were required for these experiments. The mBMMCs were prepared from a 6-week-old male mouse (Japan SLC Inc., Shizuoka, Japan) according to a previously described method [4,6]. Briefly, bone marrow cells were cultured in RPMI-1640 medium (Sigma) supplemented with 10% heat-inactivated fetal calf serum (Equitech-Bio, Kerrville, TX), 10 μ M 2-mercaptoethanol (Wako, Osaka, Japan), 20 mM Hepes buffer (Sigma), 1 mM sodium pyruvate (Sigma), 100 μ M MEM non-essential amino acids (Sigma), 2 μ g/ml gentamicin solution (Sigma), 20 μ l/ml penicillin–streptomycin solution stabilized (Sigma), 20 ng/ml recombinant murine interleukin-3 (IL-3; Peprotech, Rocky Hill, NJ), 40 ng/ml recombinant murine SCF (Peprotech), 5 ng/ml recombinant murine IL-9 (R&D, Minneapolis, MN) and 1 ng/ml TGF- β 1 (Sigma) at 37 °C in a humidified 5% CO $_2$ atmosphere. Mutant *TRPA1*-null mice [7] were generously provided by Dr. David Julius (University of California, San Francisco). The mBMMCs were generated from BALB/c mice, *TRPA1*^{-/-} C57BL/6 mice and *TRPA1*^{+/-} C57BL/6 mice. All of the animal care and experiments were approved by the Animal Experiment Committee at University of Toyama (Authorization No. S-2009 INM-9).

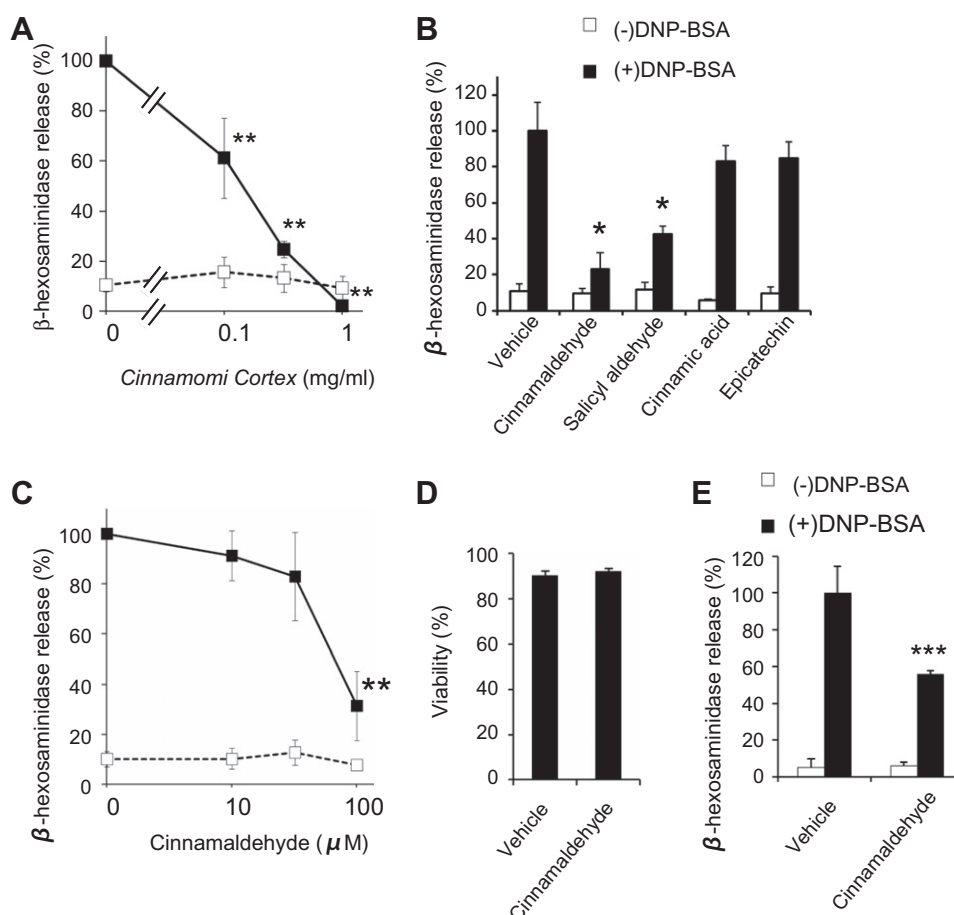


Fig. 1. *C. cortex* extracts and cinnamaldehyde inhibit the degranulation of mBMMCs and RBL-2H3 cells. mBMMCs sensitized with anti-DNP IgE were treated with *C. cortex* extracts (A) or cinnamaldehyde (C) at the concentrations noted or with 100 μ M of cinnamaldehyde, salicyl aldehyde, cinnamic acid, and epicatechin (B) for 30 min. (D) mBMMCs were treated with vehicle or 100 μ M cinnamaldehyde for 1.5 h and stained with propidium iodide. Cell viability were determined by flow cytometry (E) RBL-2H3 cells sensitized with anti-DNP IgE were treated with 100 μ M cinnamaldehyde for 30 min. Cells were stimulated with (filled) or without (open) 100 ng/ml DNP-BSA for 1 h, and the β -hexosaminidase release was determined. Data are represented as the mean $\pm$ S.D. for 3–5 experiments. * P < 0.05, ** P < 0.01, *** P < 0.001, as compared with the vehicle-treated cells.

2.3. Degranulation assay

The degree of degranulation was assessed by measuring β -hexosaminidase release, as previously described [4,6]. The mBMMCs were treated with test drugs for 30 min and stimulated with antigen-IgE.

2.4. Cell viability measurement

The mBMMCs were treated with vehicle or 100 μ M cinnamaldehyde for 1.5 h and stained with 2 μ g/ml propidium iodide (Sigma). Viability of the cells were measured by flow cytometric analysis using FACSCalibur and Cell QuestPro software version 6.0 (BD Biosciences, Franklin Lakes, NJ).

2.5. Intracellular calcium imaging

The mBMMCs, sensitized with 1.5 μ g/ml mouse anti-DNP IgE antibodies, were loaded with 10 μ M fluo 3-AM (Dojindo, Kumamoto, Japan) in loading buffer (118 mM NaCl, 4.7 mM KCl, 1 mM Na_2HPO_4 , 1.13 mM MgCl_2 , 10 mM HEPES, 5.5 mM D-glucose,

100 mM L-glutamine, 1.3 mM CaCl_2 , 2% MEM non-essential amino acids, and 0.2% BSA) for 30 min. The sensitized mBMMCs were then washed once, resuspended in loading buffer with vehicle or 100 μ M cinnamaldehyde, plated in a 6-cm dish containing glass coverslips coated with CellTak (BD Biosciences), incubated for 10 min, and then observed under the microscope. The intracellular Ca^{2+} mobilization was monitored using a fluorescence microscope (IX71 system; Olympus, Tokyo, Japan) equipped with a band-pass filter set (NIBA; Olympus). The emission signal was recorded with a CCD camera (MiCAM02, BrainVision Inc., Tokyo, Japan). The changes in the dye signal were recorded and analyzed using BV-analyzer software (BrainVision, Inc.). The time courses of the fluorescence level of particular cells are expressed as the change in the fluorescence intensity from the baseline level (ΔF) normalized to the baseline-level fluorescence (F_0).

2.6. Intracellular calcium measurements

The mBMMCs were loaded with 5 μ M fura 2-AM (Dojindo) in loading buffer with or without Ca^{2+} for 30 min, washed once, resuspended in 1.3 ml of loading buffer with vehicle or 100 μ M

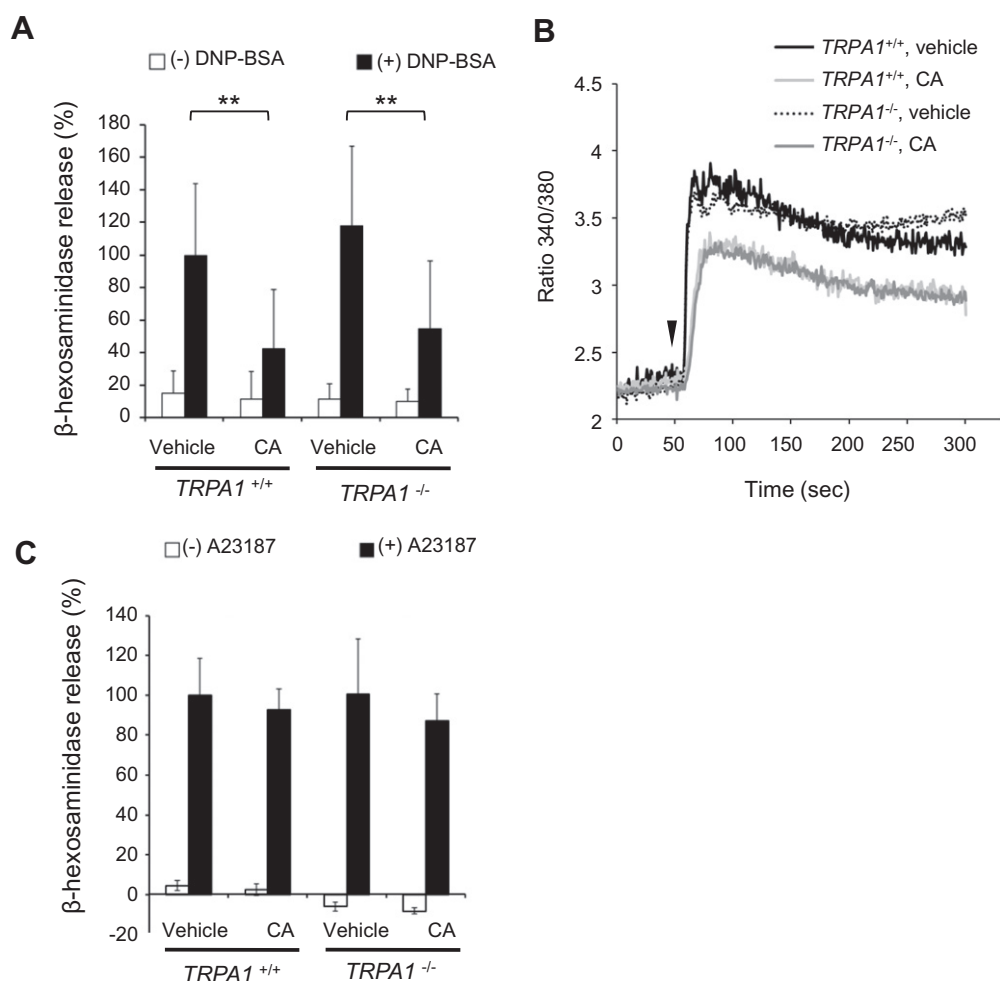


Fig. 2. Cinnamaldehyde inhibits degranulation and $[\text{Ca}^{2+}]_i$ elevation in mBMMCs derived from *TRPA1*^{-/-} mice. (A) mBMMCs derived from *TRPA1*^{+/+} (left) and *TRPA1*^{-/-} mice (right) were sensitized with anti-DNP IgE, treated with 100 μ M cinnamaldehyde (CA) or vehicle for 30 min and stimulated with (filled) or without (open) 100 ng/ml DNP-BSA for 1 h. Data are represented as the mean $\pm$ S.D. for 14–18 experiments. $^{**}P < 0.01$, as compared with vehicle-treated cells. (B) mBMMCs derived from *TRPA1*^{+/+} and *TRPA1*^{-/-} mice were sensitized with anti-DNP IgE, loaded with fura 2-AM and treated with vehicle or 100 μ M cinnamaldehyde (CA) for 30 min. These cells were then stimulated with 100 ng/ml DNP-BSA (triangle) 50 s after the monitoring began. (C) mBMMCs were incubated with 100 μ M cinnamaldehyde (CA) or vehicle for 30 min. The cells were then stimulated with (filled) or without (open) 1 μ M calcium ionophore A23187 for 20 min. The release of β -hexosaminidase was determined. Data are represented as the mean $\pm$ S.D. for 4 experiments.

cinnamaldehyde, incubated at 37 °C for 30 min, and placed in a stirred cuvette. The fluorescence measurements were performed as previously described using a fluorescence spectrophotometer intracellular Ca^{2+} measurement system (Hitachi, Tokyo, Japan) [4].

2.7. Western blot

The RBL-2H3 cells were sensitized with 200 ng/ml anti-DNP IgE antibodies overnight, pretreated with vehicle or 100 μM cinnamaldehyde for 30 min at 37 °C and then stimulated with 100 ng/ml DNP-BSA for 10 min. Whole-cell lysates were separated on a 10% (for Lyn) or 7.5% (for PLC γ 1) SDS-PAGE gel and transferred to a nitrocellulose membrane. The membranes were first incubated in blocking solution (5% BSA) and then incubated with anti-Lyn, anti-phospho-specific Lyn, anti-PLC γ 1, or anti-phospho-specific

PLC γ 1 antibodies overnight. After washing, the membrane was incubated with an HRP-conjugated secondary antibody directed against the primary antibody and then visualized using an ECL Western blot detection kit (Amersham Pharmacia Biotech, Piscataway, NJ). Densities of the immunoreactive bands were evaluated using ATTO Densitograph Software Library CS analyzer (ATTO instruments, Tokyo, Japan). Densitometric values of phospho-protein bands were normalized against the corresponding total protein.

2.8. Statistical analyses

The data are expressed as the mean $\pm$ S.D. The statistical comparisons were made using a one-way ANOVA, followed by Dunnett's multiple comparisons or student's *t*-test. A probability value (*P*) of <0.05 was considered statistically significant.

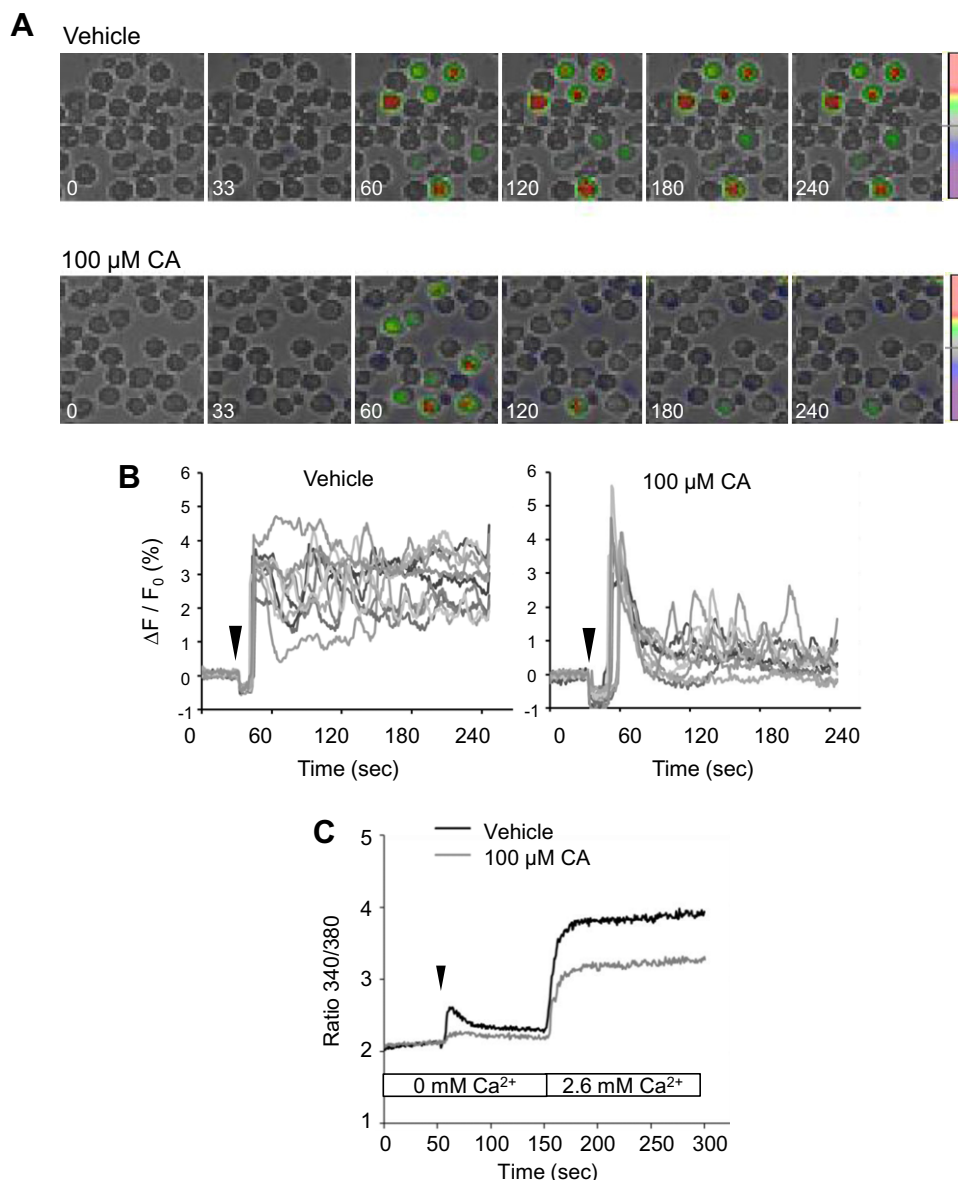


Fig. 3. Antigen-induced $[\text{Ca}^{2+}]_i$ elevation in mBMMCs is suppressed by cinnamaldehyde. (A) mBMMCs were sensitized with IgE, loaded with fluo 3-AM, treated with vehicle or 100 μM cinnamaldehyde (CA) for 10 min, and stimulated with 100 ng/ml DNP-BSA 33 s after the monitoring began. The Ca^{2+} mobilization in individual mBMMCs was monitored using a fluorescence microscope. (B) Fluorescence intensities ($\Delta F/F_0$) in response to 100 ng/ml DNP-BSA (triangle). Fluorescence intensities in 10 representative cells are shown. (C) The time course of the $[\text{Ca}^{2+}]_i$ changes in mBMMCs suspended in a stirred cuvette is shown. mBMMCs were sensitized with anti-DNP IgE, loaded with fura 2-AM and then treated with vehicle or 100 μM cinnamaldehyde (CA) for 30 min. Ca^{2+} release in the sensitized mBMMCs was elicited by treatment with 100 ng/ml DNP-BSA (triangle) in an extracellular Ca^{2+} -free environment. Ca^{2+} influx was then induced by restoring the extracellular Ca^{2+} concentration to 2.6 mM. $[\text{Ca}^{2+}]_i$ was measured as the ratio of fluorescence measured when excited by 340 and 380 nm of light.

3. Results and discussion

3.1. *Cinnamomi cortex* and cinnamaldehyde inhibit the degranulation of mBMMCs and RBL-2H3

The inhibitory effect of the *C. cortex* extract was examined in the mBMMCs. The sensitized mBMMCs were treated with the *C. cortex* extracts, and the degree of antigen-induced degranulation was assessed by β -hexosaminidase release. As shown in Fig. 1A, $\geq 100 \mu\text{g/ml}$ of *Cinnamomi cortex* extract significantly inhibited the degranulation of the mBMMCs.

We next examined whether the major constituents of *Cinnamomi cortex* (cinnamaldehyde, salicyl aldehyde, cinnamic acid, and epicatechin) inhibited the degranulation of the mBMMCs. Among these four constituents, $100 \mu\text{M}$ cinnamaldehyde showed a significant inhibitory effect, the most potent of all of the constituents, on the degranulation of the mast cells (Fig. 1B and C). *Cinnamomi cortex* contains 1–3% oil, and cinnamaldehyde constitutes approximately 90% of the oil [8]. In the present study, we used cinnamaldehyde at $100 \mu\text{M}$, which was nontoxic and comparable to the concentrations used in other in vitro studies [9]. In fact, treatment with $100 \mu\text{M}$ cinnamaldehyde for 1.5 h was not toxic to the mBMMCs (Fig. 1D). The inhibitory effect of $100 \mu\text{M}$ cinnamaldehyde on degranulation was also confirmed using RBL-2H3 cells (Fig. 1E).

3.2. Cinnamaldehyde inhibition of the antigen-triggered response of mBMMCs is *TRPA1* independent

Cinnamaldehyde is a well-known agonist of *TRPA1*, a member of the transient receptor potential (TRP) ion channel family [9]. To determine whether *TRPA1* contributed to the response of mucosal mast cells to cinnamaldehyde, mBMMCs were generated from mice lacking functional *TRPA1* channels; contrary to our expectation, cinnamaldehyde inhibited the antigen-IgE-induced degranulation of the *TRPA1*^{−/−} mBMMCs and *TRPA1*^{+/+} mBMMCs (Fig. 2A). The intracellular free, ionized calcium concentration ($[\text{Ca}^{2+}]_i$) was also measured using an intracellular Ca^{2+} measurement system that uses a fluorescence spectrophotometer and fura 2, a fluorescent indicator. Cinnamaldehyde inhibited the antigen-IgE-induced elevation of $[\text{Ca}^{2+}]_i$ in the *TRPA1*^{−/−} mBMMCs and *TRPA1*^{+/+} mBMMCs (Fig. 2B). These results indicate that the effects of cinnamaldehyde are *TRPA1*-independent. In addition, cinnamaldehyde did not affect calcium ionophore A23187-induced degranulation in both *TRPA1*^{−/−} and *TRPA1*^{+/+} mBMMCs (Fig. 2C), suggesting that the action of cinnamaldehyde was specifically restricted to Fc ϵ RI-proximal signals.

3.3. Cinnamaldehyde inhibits the $[\text{Ca}^{2+}]_i$ elevation induced by antigen-IgE stimulation

The intracellular Ca^{2+} mobilization in mast cells upon antigen-IgE stimulation is divided into two parts: a fast and transient Ca^{2+} release from the intracellular stores and a sustained influx of extracellular Ca^{2+} mediated by STIM1 and store-operated Ca^{2+} channels across the plasma membrane [10,11]. Because the antigen-IgE-mediated Ca^{2+} mobilization and degranulation is completely abolished in extracellular Ca^{2+} -free conditions, Ca^{2+} influx is, therefore, critical for mast cell activation and degranulation [12]. An intracellular calcium imaging system allowed us to examine the Ca^{2+} signals simultaneously in many individual cells with both high temporal and spatial resolution. Fluo 3, a fluorescent indicator, was loaded into the mBMMCs that were stimulated with antigen, and the fluorescence intensity of individual cells was examined using the imaging system. Consistent with previous findings [10], the vehicle-treated cells showed a sharp Ca^{2+} spike,

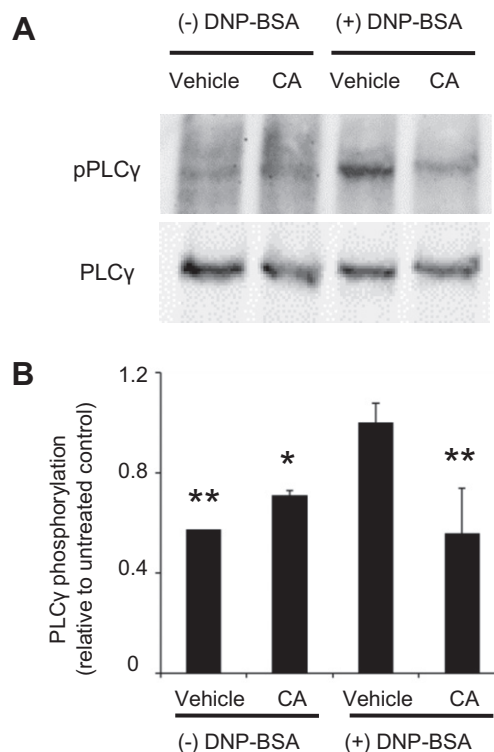


Fig. 4. Cinnamaldehyde inhibits the tyrosine phosphorylation of PLC γ 1 in antigen-stimulated RBL-2H3 cells. RBL-2H3 cells were sensitized with 200 ng/ml anti-DNP IgE and treated with $100 \mu\text{M}$ cinnamaldehyde (CA) or vehicle for 30 min prior to stimulation with or without 100 ng/ml DNP-BSA for 10 min. Total cell lysates were immunoblotted with antibodies against PLC γ 1 and activated PLC γ 1 (pPLC γ). (A) Representative data of three independent experiments. (B) The PLC γ 1 phosphorylation levels were determined by densitometry and normalized against PLC γ 1 protein levels and then the phosphorylation levels in untreated control (vehicle-treated and unstimulated cells). Data are represented as the mean $\pm$ S.D. for 3 experiments. * $P < 0.05$, ** $P < 0.01$ compared with vehicle-treated stimulated cells.

followed by a sustained Ca^{2+} plateau that was often accompanied oscillations (Fig. 3A and B). Cinnamaldehyde ($100 \mu\text{M}$) inhibited only the sustained Ca^{2+} responses and not the initial Ca^{2+} peak triggered by the antigen, suggesting that cinnamaldehyde only inhibits the Ca^{2+} influx and not the Ca^{2+} release from intracellular stores.

To assess the Ca^{2+} release from the intracellular Ca^{2+} stores and the Ca^{2+} influx independently and quantitatively, the $[\text{Ca}^{2+}]_i$ in antigen-stimulated cells was measured in the absence of extracellular Ca^{2+} , and then 2.6 mM Ca^{2+} was added to the cells (Fig. 3C). We noted a considerable suppression of the Ca^{2+} influx in the cinnamaldehyde-treated cells. These results are similar to the results seen using STIM1-deficient mouse-derived mast cells [11].

3.4. Cinnamaldehyde inhibits the antigen-induced phosphorylation of PLCγ1

The antigen-induced crosslinking of cell surface Fc ϵ RI triggers the activation of Lyn, a member of the Src family of protein tyrosine kinases [13]. The activation of Lyn leads to a signaling cascade that results in the phosphorylation of PLC γ , which, in turn, yields inositol 1,4,5-trisphosphate to induce the liberation of $[\text{Ca}^{2+}]_i$. It has been reported that the initial peak of $[\text{Ca}^{2+}]_i$ is caused by the sequential activation of phospholipase D (PLD) and sphingosine kinase (SPHK), whereas the sustained entry of Ca^{2+} is dependent of the activation of PLC γ [14]. Because cinnamaldehyde inhibits only the latter Ca^{2+} influx, we focused on PLC γ and examined the effect of cinnamaldehyde on the phosphorylation of PLC γ 1. As shown in Fig. 4, the result of our western blot analysis revealed that

cinnamaldehyde significantly inhibited the phosphorylation of PLC γ 1 ($55.8 \pm 17.9\%$ compared to vehicle treatment in the stimulated cells; $n = 3$). In contrast, the phosphorylation of Lyn was not inhibited by cinnamaldehyde ($113.0 \pm 22.0\%$ compared to vehicle treatment in the stimulated cells; $n = 3$, data not shown).

In conclusion, our findings in the present study revealed that cinnamaldehyde inhibited mucosal mast-cell activation by suppressing PLC γ 1 signaling. Consistent with our results, a recent study has shown that cinnamaldehyde has an inhibitory effect on PLC signaling in human embryonic kidney (HEK) 293T cells in a TRPA1-independent manner [15]. Although further experiments will be required to identify the direct target of cinnamaldehyde on mast cells, our results suggest a therapeutic potential for cinnamaldehyde as an inhibitor of the antigen-stimulated $[Ca^{2+}]_i$ elevation and degranulation of mucosal mast cells. Because cinnamaldehyde is a food product, it is expected that toxicity in vivo would be minimal. Our findings may allow the development of potent therapeutic agents against allergic diseases related to mucosal mast cells, such as food allergies, based on the novel mechanism of interfering with PLC γ 1 signaling in mucosal mast cell activation.

Acknowledgments

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References

- [1] J. Rivera, A.M. Gilfillan, Molecular regulation of mast cell activation, *Journal of Allergy and Clinical Immunology* 117 (2006) 1214–1225.
- [2] L.C.H. Yu, M.H. Perdue, Role of mast cells in intestinal mucosal function: studies in models of hypersensitivity and stress, *Immunological Reviews* 179 (2001) 61–73.
- [3] T. Yamamoto, K. Fujiwara, M. Yoshida, N. Kageyama-Yahara, H. Kuramoto, N. Shibahara, M. Kadowaki, Therapeutic effect of kakkonto in a mouse model of food allergy with gastrointestinal symptoms, *International Archives of Allergy and Immunology* 148 (2009) 175–185.
- [4] N. Kageyama-Yahara, Y. Suehiro, F. Maeda, S.-i. Kageyama, J. Fukuoka, T. Katagiri, T. Yamamoto, M. Kadowaki, Pentagalloylglucose down-regulates mast cell surface Fc ϵ RI expression in vitro and in vivo, *FEBS letters* 584 (2010) 111–118.
- [5] M. Kubo, S. Ma, J. Wu, H. Matsuda, Anti-inflammatory activities of 70% methanolic extract from Cinnamomi Cortex, *Biological and Pharmaceutical Bulletin* 19 (1996) 1041–1045.
- [6] N. Kageyama-Yahara, Y. Suehiro, T. Yamamoto, M. Kadowaki, IgE-induced degranulation of mucosal mast cells is negatively regulated via nicotinic acetylcholine receptors, *Biochemical and Biophysical Research Communications* 377 (2008) 321–325.
- [7] D.M. Bautista, S.-E. Jordt, T. Nikai, P.R. Tsuruda, A.J. Read, J. Poblete, E.N. Yamoah, A.I. Basbaum, D. Julius, TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents, *Cell* 124 (2006) 1269–1282.
- [8] Y. Shiraga, K. Okano, T. Akira, C. Fukaya, K. Yokoyama, S. Tanaka, H. Fukui, M. Tabata, Structures of potent antiulcerogenic compounds from, *Tetrahedron* 44 (1988) 4703–4711.
- [9] M. Bandell, G.M. Story, S.W. Hwang, V. Viswanath, S.R. Eid, M.J. Petrus, T.J. Earley, A. Patapoutian, Noxious Cold Ion Channel TRPA1 Is Activated by Pungent Compounds and Bradykinin, *Neuron* 41 (2004) 849–857.
- [10] P. Millard, T. Ryan, W. Webb, C. Fewtrell, Immunoglobulin E receptor cross-linking induces oscillations in intracellular free ionized calcium in individual tumor mast cells, *The Journal of Biological Chemistry* 264 (1989) 19730–19739.
- [11] Y. Baba, K. Nishida, Y. Fujii, T. Hirano, M. Hikida, T. Kurosaki, Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses, *Nature Immunology* 9 (2008) 81–88.
- [12] K. Nishida, S. Yamasaki, Y. Ito, K. Kabu, K. Hattori, T. Tezuka, H. Nishizumi, D. Kitamura, R. Goitsuka, R.S. Geha, T. Yamamoto, T. Yagi, T. Hirano, Fc ϵ RI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane, *The Journal of Cell Biology* 170 (2005) 115–126.
- [13] J. Kalesnikoff, S.J. Galli, New developments in mast cell biology, *Nature Immunology* 9 (2008) 1215–1223.
- [14] A.J. Melendez, A.K. Khaw, Dichotomy of Ca^{2+} signals triggered by different phospholipid pathways in antigen stimulation of human mast cells, *Journal of Biological Chemistry* 277 (2002) 17255–17262.
- [15] K.Y. Kim, S. Bang, S. Han, Y.H. Nguyen, T.M. Kang, K.W. Kang, S.W. Hwang, TRP-independent inhibition of the phospholipase C pathway by natural sensory ligands, *Biochemical and Biophysical Research Communications* 370 (2008) 295–300.