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Morin inhibits Fyn kinase in mast cells and IgE-mediated type I hypersensitivity response in vivo

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ABSTRACT

Mast cells are responsible for IgE-mediated allergic responses. Although dietary flavonoid morin has been known to suppress mast cell activation, its in vivo anti-allergic activity and the underlying mechanisms remain are largely unknown. In this study, we determine whether morin suppresses IgE-mediated allergic responses in an animal model and its mechanism of action. Morin suppressed IgE-mediated PCA in mice (ED_{50} 23.9 mg/kg) and inhibited degranulation and production of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-4 in antigen (Ag)-stimulated mast cells. The mechanism of action was a follows. Morin inhibited the activating phosphorylation of spleen tyrosine kinase (Syk) and linker for activation of T cells (LAT) in rat basophilic leukemia (RBL)-2H3 cells and bone marrow-derived mast cells (BMMCs). Akt and the mitogen-activated protein (MAP) kinases, p38, extracellular signal-regulated kinase (ERK)1/2, and c-Jun N-terminal kinase (JNK) were inhibited as well. In vitro kinase assay indicated that Fyn kinase, not Lyn and Syk, was inhibited by morin in a dose-dependent manner (IC_{50} 5.7 μM). In conclusion, the results suggest that morin suppresses the IgE-mediated allergic response by primarily inhibiting Fyn kinase in mast cells.

1. Introduction

Morin is a flavonoid that has been identified in fruits, vegetables, tea, wine, and many Oriental medicinal herbs [1]. Morin, [2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one] (Fig. 1A), is a yellow pigment isolated from plants of the Moraceae family [2]. It exhibits a variety of pharmacological activities such as anti-inflammatory activity [3,4], anti-oxidant activity [5,6], and an anti-tumor promotion effect [7,8]. Although morin inhibits the degranulation of mast cells [9,10], the in vivo anti-allergic activity and its mechanism of action is largely unknown.

The number of patients suffering allergic diseases has increased dramatically over the past decades. An estimated 20% of the population in developed countries suffers from allergies at some time in their life. Allergic diseases are mediated by the expansion of the T helper 2 cell subset of T cells and the isotype switching of B cells to generate IgE antibodies toward specific environmental allergens [11]. The aggregation of IgE and FcεRI on mast cells initiates the allergic responses. The manifestations vary according to the sites of access of allergen: the skin (atopic dermatitis or eczema), nose (rhinitis), lungs (asthma) and gut (food allergic reactions) [12,13]. Antigen-crosslinking of the IgE-FcεRI complexes on mast cells results in degranulation and the synthesis of lipid mediators to induce the early phase of the allergic reaction [14,15].

Approaches to the treatment of allergic diseases include treatment with glucocorticoids and β_2-adrenoceptor antagonists, allergen-specific immunotherapy, humanized anti-IgE Ab administration, DNA vaccination, cytokine-based immunotherapies, and the use of antagonists of leukotriene and histamine receptors [16]. However, certain difficulties and side effects are associated with these therapies. For example, there is a long list of undesirable side effects associated with corticotherapy, such as alterations in fat

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Abbreviations: RBL, rat basophilic leukemia; BMMC, bone marrow-derived mast cell; PCA, passive cutaneous anaphylaxis; ITAM, immunoreceptor tyrosine-based activation motif; Syk, spleen tyrosine kinase; LAT, linker for activation of T cells; Gab2, Grb2-associated binder 2; MAP kinase, mitogen-activated protein kinases; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; DNP-BSA, dinitrophenol-conjugated bovine serum albumin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PIPES, 1,4-piperazinediethanesulfonic acid.

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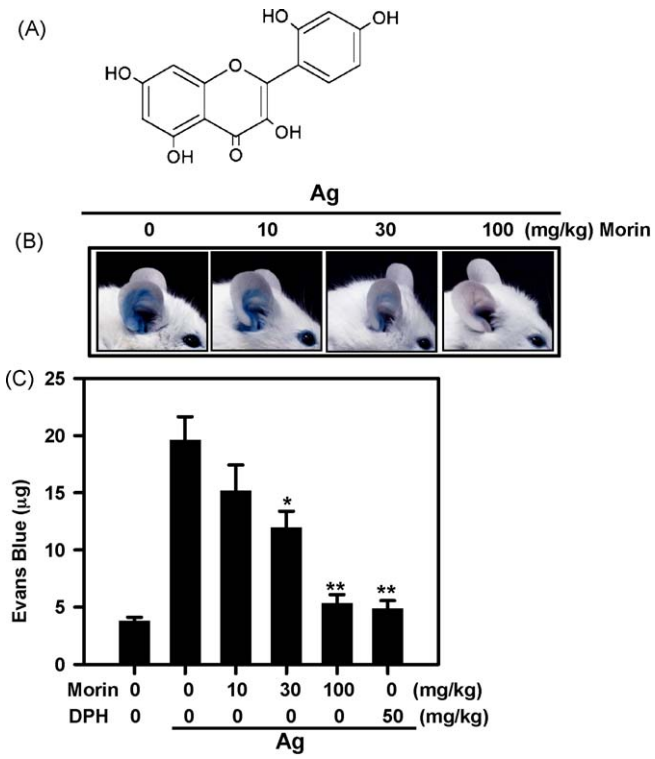

Fig. 1. Effect of morin on IgE-mediated passive cutaneous anaphylaxis (PCA). (A) Molecular structure of morin. (B–C) The anti-allergic effect was assessed using PCA mouse model. Balb/c mice were intravenously injected with a 250-μg antigen (DNP-BSA) containing 4% Evans blue 24 h after intradermal administration of a dinitrophenol (DNP)-specific IgE antibody (0.5 μg) into a mouse ear. Morin (10–100 mg/kg) was orally administrated 1 h before an administration of antigen. The dye extravasated by antigen was extracted from the ear and the amount was measured by absorbance. The representative photograph of ears (B) and the mean ± S.E.M. of values (C) from three independent experiments, each with 10 mice, are shown. The asterisks indicate significant difference from Ag-stimulated controls without morin ($^{*}p < 0.05$; $^{**}p < 0.01$). Diphenylhydramine (DPH, 50 mg/kg) was used as a typical anti-histamine reference drug.

distribution, gastric irritation, and peptic ulcer [17]. Desensitizing immunotherapy is effective but complicated and occasionally hazardous. Anti-IgE Ab administration may be another good option, but it is very expensive and only partially effective [18].

Given the critical role played by mast cells in the induction of the allergic response, inhibitors of critical enzymes involved in mast cell activation might be good treatment options [18]. The Src-family kinases Lyn, Fyn, and spleen tyrosine kinase (Syk) are important because of their roles in regulating degranulation and allergic cytokine production during the IgE-mediated activation of mast cells [15]. For example, the Syk inhibitor R112 exhibited some efficacy in seasonal allergic rhinitis patients [19]. Curcumin was identified as a Syk inhibitor that inhibits the type I hypersensitive reaction in mice [20].

In this study, we describe the anti-allergic activity of morin in an animal model and its mechanism of action in vitro and in mast cells. Our results indicate that morin reversibly suppresses the degranulation and secretion of inflammatory cytokines through the inhibition of mast cells at the level of Src-family kinases, most probably Fyn kinase.

2. Materials and methods

2.1. Reagents

Reagents were obtained from the following sources. Morin, PP2, diphenylhydramine (DPH), thapsigargin, and ionomycin from Calbiochem (La Jolla, CA); Evans blue, dinitrophenol-conjugated bovine serum albumin (DNP-BSA), and DNP-specific monoclonal IgE from Sigma–Aldrich (St. Louis, MO); Abs against the phosphorylated forms of extracellular signal-regulated kinase (ERK)1/2, p38, c-Jun N-terminal kinase (JNK), Akt, Syk (Y317 in murine), linker for activation of T cells (LAT) (Y191 in humans) from Cell Signaling Technology Inc. (Danvers, MA); Abs against Syk and actin from Santa Cruz Biotechnology Inc. (Santa Cruz, CA); and Ab (4G10) against phosphotyrosine (pY) and Abs against LAT from Upstate Biotechnology (Lake Placid, NY). Enzyme-linked immunosorbant assay (ELISA) kits from Invitrogen-Biosource Cytokine & Signaling (Camarillo, CA) were used to measure the TNF-α and IL-4 levels in culture media. Cell-culture reagents were purchased from GIBCO/Life Technologies Inc. (Rockville, MD).

2.2. Animals

Male Balb/c mice (aged 4 weeks) were obtained from the Dae Han Experimental Animal Center (Eumsung, Korea), and they were housed in the animal facilities at the College of Medicine in Konkuk University. Ten mice were placed in each cage in a laminar airflow cabinet where a temperature of 22 ± 1 °C and a relative humidity of 55 ± 10% were maintained throughout the study. The animal study was performed in accordance with the institutional guidelines. The protocol had received prior approval from the Institutional Animal Care and Use Committee (IACUC) at Konkuk University.

2.3. Induction of IgE-mediated passive cutaneous anaphylaxis (PCA) in mice

PCA was induced in mice according to a previous report [20]. Briefly, the mice were intravenously injected with 250 μg DNP-BSA (antigen, Ag) in 250 μl PBS containing 4% Evans blue 24 h after the intradermal administration of a DNP-specific IgE Ab (0.5 μg) into the ear. To measure the activity of morin, it was orally administered 1 h before Ag administration. The mice were euthanized 1 h after treatment with the Ag, and the treated ear was excised in order to measure the amount of dye extravasated by the Ag. The dye was extracted overnight from the ear in 700 μl formamide at 63 °C. The absorbance of the dye was measured at 620 nm.

2.4. Isolation and culture of bone marrow-derived mast cells (BMMCs) and rat basophilic leukemia (RBL)-2H3 cells

BMMCs were isolated according to a previous report with a minor modification [21]. Briefly, BMMCs isolated from male Balb/c mice were cultured in a medium (RPMI 1640, containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics, and 10% fetal-bovine serum (FBS)) containing 10 ng/ml IL-3. The BMMCs were used 3–4 weeks after their isolation. RBL-2H3 cells were grown as monolayers in minimum essential medium (MEM) with Earle's salts, supplemented with glutamine, antibiotics, and 15% FBS.

2.5. Stimulation and measurement of degranulation in BMMCs and RBL-2H3 cells

The RBL-2H3 cells were transferred to a 24-well plate (2×10^5 cells per well), and the BMMCs were transferred to a microtube (2×10^5 cells per tube). Next, the cells were incubated overnight in a complete medium containing 50 ng/ml DNP-specific IgE. The cultures were washed, and a buffered solution was added (0.2 ml/well). Experiments were performed using intact RBL-2H3 cells in 1,4-piperazinediethanesulfonic acid (PIPES)-buffered medium (25 mM PIPES, pH 7.2, 159 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 5.6 mM glucose, and 0.1% fatty-acid-free fraction V from a bovine serum) and using intact BMMCs in a Tyrode buffer

(20 mM HEPES, pH 7.4, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, and 0.05% BSA). The cells were incubated for 30 min with or without morin before adding 25 ng/ml of the Ag for 10 min or for indicated times. Degranulation was determined by measuring the activity of β-hexosaminidase, a granule marker, in culture media. The release of *p*-nitrophenol from *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide was measured [22].

2.6. Western blot analysis and immunoprecipitation

The Western blot analysis and immunoprecipitation (IP) were performed by the same protocol as our previous report with a minor modification [20]. Cells were stimulated with 25 ng/ml Ag for 7 min or as indicated, chilled with ice to stop stimulation, and then the cells were washed twice with ice-cold 1× PBS and then lysed (30 min) in either 0.25 ml lysis buffer for six-well plates or in 0.5 ml lysis buffer for 100 m/m dishes. The lysis buffers contained the following: 20 mM HEPES, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 10% glycerol, 60 mM octyl β-glucoside, 10 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 2.5 mM nitrophenylphosphate, 0.7 μg/ml pepstatin, and a protease-inhibitor cocktail tablet. For immunoprecipitation, the supernatant fraction was "precleared" by adding 50 μl protein G-agarose, followed by gentle rocking for 1 h and centrifugation. Supernatant fractions containing equal amount of protein were used for immunoprecipitation. Syk and LAT were immunoprecipitated by overnight incubation (at 4 °C with gentle rocking) with specific Abs and then with protein G-agarose. The agarose was washed five times with a washing buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 0.1% Nonidet P-40, 10% glycerol, 10 mM NaF, 1 mM Na_3VO_4, 1 mM PMSF, 2.5 mM nitrophenylphosphate, 0.7 μg/ml pepstatin, and protease-inhibitor cocktail tablet), and the precipitates were denatured by boiling at 95 °C for 5 min in a 2× Laemmli buffer [23]. For immunoblotting, proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Blots were probed with the indicated primary Abs and peroxidase-labeled secondary Abs and visualized using chemiluminescence.

2.7. Extraction of RNA and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from the RBL-2H3 cells using Trizol Reagent (Invitrogen, Carlsbad, CA) and reverse transcribed using the Superscript first-strand synthesis system (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The polymerase chain reaction was performed at 94 °C for 45 s, at 55 °C for 45 s, and at 72 °C for 60 s, for 30 cycles. The following primers were used: rat TNF-α forward 5′-CACCACGCTCTTCTGTCTACTGAAC-3′; rat TNF-α reverse: 5′-CCGGACTCCGTGATGTCTAAGTACT-3′; rat IL-4 forward 5′-ACCTTGCTG TCACCCTGTTC-3′; rat IL-4 reverse 5′-TTGTGAGCGTGGACTCATTC-3′; rat glyceraldehydes-3-phosphate dehydrogenase forward 5′-GTGGAGTCTACTGGCG TCTTC-3′; rat glyceraldehydes-3-phosphate dehydrogenase reverse: 5′-CCAAGGC TGTGGGCA AGGTCA-3′.

2.8. ELISA of TNF-α and IL-4

IgE-primed RBL-2H3 cells were stimulated with 25 ng/ml DNP-BSA for 4 h, with or without morin. The levels of TNF-α or IL-4 secreted in culture media were measured by using ELISA kits according to the manufacturer's instructions (Invitrogen-Biosource Cytokine & Signaling, Camarillo, CA).

2.9. In vitro tyrosine kinase assay

The in vitro tyrosine kinase assay was conducted by the manufacturer's instructions (Millipore Corp., Billerica, MA). Briefly, in a final reaction volume of 25 μl, each human tyrosine kinase (Lyn, Fyn, or Syk) (5–10 mU) was incubated with 50 mM Tris, pH 7.5, 0.1 mM EGTA, 0.1 mM Na_3VO_4, 10 mM Mg Acetate and [γ-^{33}P-ATP] (specific activity approximately 500 cpm/pmol). The reaction buffers contained 250 μM Cdc2 peptide (KVEKIGEGTYGVVYK) as a Fyn substrate or 0.1 mg/ml poly(Glu, Tyr) 4:1 as a substrate for assay of Lyn and Syk, respectively. The reaction was initiated by the addition of the MgATP mix. After incubation for 40 min at room temperature, the reaction was stopped by the addition of 5 μl of a 3% phosphoric acid solution. The reaction mixture (10 μl) was then spotted onto a Filtermat A and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

2.10. Statistical analysis

The results were presented as mean ± S.E.M. from three or more independent experiments. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Dunnett's test. All statistical calculations ($^{*}p < 0.05$ and $^{**}p < 0.01$) were performed using SigmaStat software (Systat Software, Inc.; Point Richmond, CA).

3. Results

3.1. Effect of morin on IgE-mediated PCA reaction in mice

On the basis of previous reports that morin inhibits degranulation of Ag-stimulated mast cells [9,10], we first measured its anti-allergic activity in mice. IgE-mediated PCA was successfully induced by the sequential injection of IgE and DNP-BSA in mice, and the PCA reactions were suppressed by morin in a dose-dependent manner (Fig. 1B and C, ED_{50} 23.9 mg/kg). The effect of morin was evident at a dose of 10 mg/kg. Furthermore, nearly complete suppression of the Ag-induced PCA reaction was achieved with 100 mg/kg morin. Morin's effect was equivalent to that of 50 mg/kg diphenylhydramine (DPH), a typical anti-histamine drug.

3.2. Effect of morin on Ag-stimulated degranulation in RBL-2H3 cells and BMMCs

Secretion of preformed allergic mediators such as histamine and various proteases in granules is a critical part of the local allergic reaction. Therefore, we tested whether morin inhibited Ag-stimulated degranulation by using two types of mast cells: RBL-2H3 cells and BMMCs. Morin inhibited Ag-induced degranulation in RBL-2H3 cells (Fig. 2A) and BMMCs (Fig. 2B) in a dose-dependent manner (IC_{50} 1.1 μM for RBL-2H3 cells; 3.3 μM for BMMCs). Of note, the inhibitory effect of morin on mast cell degranulation could be reversed by washing the cells with the reaction buffer after incubation with morin for 1 h, indicating that the inhibitory effect of morin is reversible (Fig. 2C).

3.3. Effect of morin on the expression and secretion of inflammatory cytokines

TNF-α and IL-4 are inflammatory cytokines that induce delayed type hypersensitive responses. The Ag-stimulated expression of TNF-α and IL-4 mRNA (Fig. 3A) and production of their protein counterparts (Fig. 3B and C) were markedly suppressed by morin in a dose-dependent manner (IC_{50} 1.9 μM for TNF-α; 0.9 μM for IL-4). Significant inhibition was observed at doses as low as 1 μM morin and nearly complete suppression was obtained at 10 μM morin in Ag-stimulated mast cells as was the case for the generic Src-family kinase inhibitor, PP2 (Fig. 3B and C).

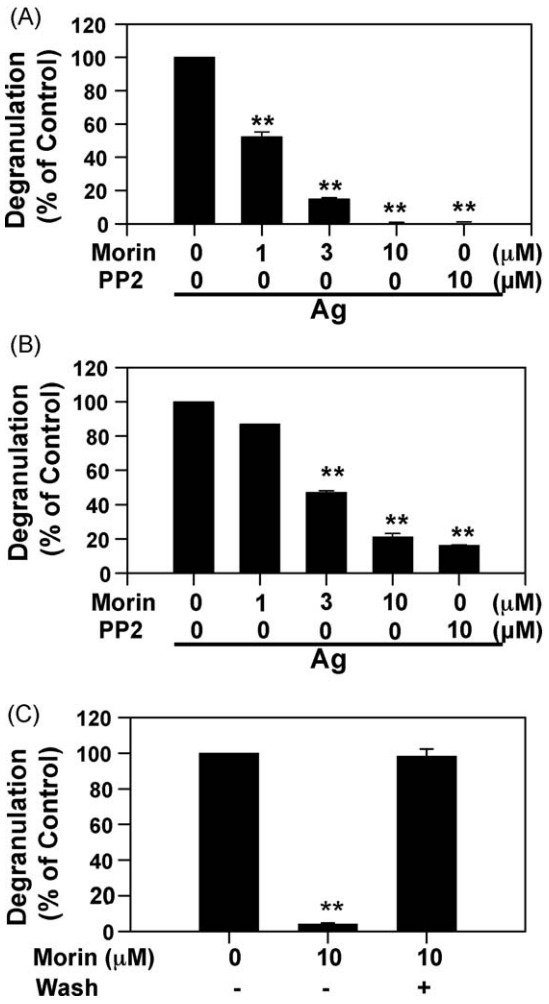

Fig. 2. Effect and reversibility of morin on Ag-stimulated degranulation in mast cells. (A–B) After priming RBL-2H3 cells (A) or BMMCs (B) with 50 ng/ml DNP-specific IgE, the cells were stimulated with 25 ng/ml antigen for 10 min. The degranulation was determined by measurement of the activity of β-hexosaminidase in culture media. (C) Reversibility of morin's effect in RBL-2H3 cells. The RBL-2H3 cells were washed out five times with PIPES buffer after incubating with 10 μM morin for 1 h and then stimulated for 10 min with antigen. The values indicate mean ± S.E.M. from three independent experiments. Significant differences with the Ag-stimulated controls without morin are indicated, $^{*}p < 0.05$ and $^{**}p < 0.01$. PP2 is a general Src-family kinase inhibitor.

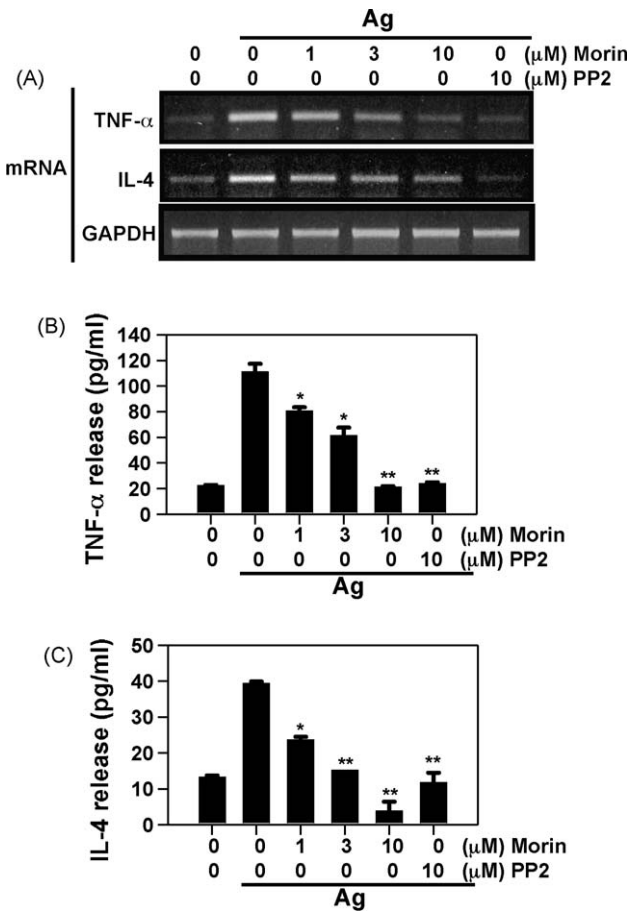

Fig. 3. Effect of morin on the expression and secretion of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-4 in RBL-2H3 mast cells. (A) IgE-primed RBL-2H3 cells were stimulated with 25 ng/ml Ag, or not stimulated. Total RNA was isolated and then reversely transcribed. Polymerase chain reaction using IL-4- or TNF-α-specific primers was performed as described in Section 2. The representative images from three independent experiments are shown. (B–C) The levels of TNF-α (B) and IL-4 (C) in the culture supernatant were determined by using commercial Enzyme-linked immunosorbent assay (ELISA) kits. Asterisks indicate significant differences from Ag-stimulated controls, which are not treated with morin, $^{*}p < 0.05$ and $^{**}p < 0.01$. PP2 is a general Src-family kinase inhibitor.

3.4. The mechanism of action of morin in mast cells

We investigated potential molecular targets that may account for the inhibitory actions of morin on mast cells. The results show that morin inhibited the activating phosphorylations of Syk and LAT in Ag-stimulated RBL-2H3 cells and BMMCs (Fig. 4). The effects of morin were dose-dependent and were evident at a dose as low as 1 μM, and the activating phosphorylation of Syk and LAT was completely blocked at 10 μM morin (Fig. 4). Next, we investigated the effects on several downstream events including phosphorylation of PLCγ1 and Akt, a surrogate for the activation of phosphotidylinositol 3-kinase, and the activating phosphorylation of the MAP kinases Erk1/2, p38, and JNK that play a role in the production of TNF-α and IL-4 [24–26]. The activating phosphorylations of PLCγ1, Akt and the MAP kinsaes were inhibited by morin in a dose-dependent manner and at similar concentrations to those required for inhibition of Syk and LAT phosphorylation (Fig. 4). At 10 μM, morin mimicked the inhibitory effects of 10 μM PP2 on all of the above phosphorylations (Fig. 4).

3.5. Effect of morin on the activity of Fyn kinase in vitro

Morin did not inhibit the degranulation of mast cells stimulated with thapsigargin or ionomycin, which act by directly mobilizing intracellular and extracellular calcium (Fig. 5). The results indicate that the effect of morin is specifically restricted to the receptor-proximal signals, such as Src-family kinases including Lyn, Fyn kinases, or Syk in IgE-mediated activation of mast cells. Syk kinase is initially phosphorylated by Lyn kinase and/or other Src-family kinases, and it is further phosphoylated by its autophosphorylation. Therefore, we tested whether morin directly inhibited one or more of the aforementioned tyrosine kinases in vitro. Interestingly, we found that morin specifically inhibited Fyn kinase (IC_{50} 5.7 μM), not Lyn and minimally Syk if any, in a dose-dependent manner at concentration that could account for the inhibition of downstream Syk-dependent events (Fig. 6).

4. Discussion

IgE-mediated allergic diseases are a significant health problem in many industrialized countries [27,28]. Current therapeutic approaches are largely ameliorative rather than curative and can cause unexpected side effects. Consequently, novel approaches are

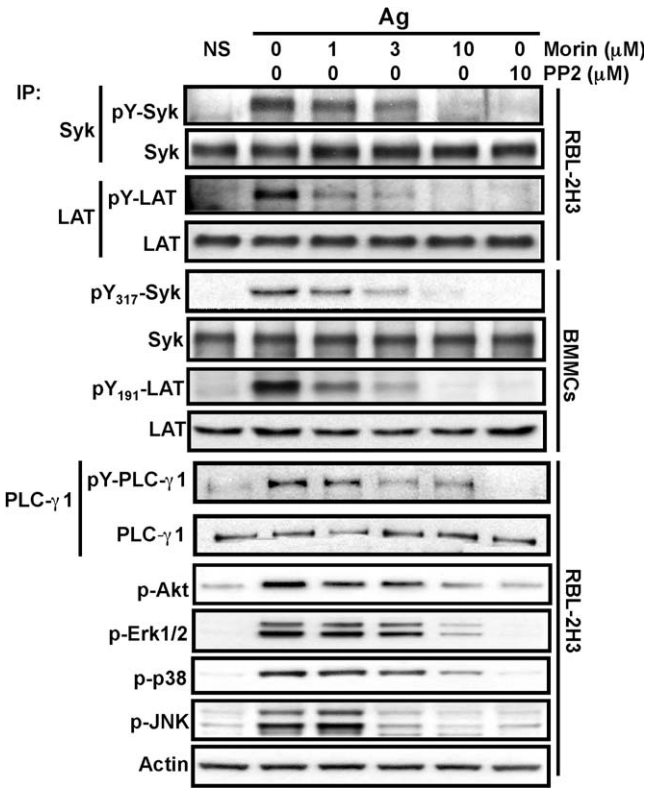

Fig. 4. Effect of morin on the activating phosphorylation of Syk and Syk-mediated downstream molecules in Ag-stimulated mast cells. The IgE-primed RBL-2H3 cells or BMMCs were stimulated with 25 ng/ml Ag (DNP-BSA), with or without morin at the indicated doses, for 7 min. Syk, LAT, and PLCγ1 were immunoprecipitated with specific Abs and then subjected to immunoblot analysis. The proteins derived from the RBL-2H3 cell lysates were subjected to immunoblot analysis to detect phosphorylated forms of Akt and three typical MAP kinases. Representative immunoblot images are shown from three independent experiments. PP2 is a general Src-family kinase inhibitor.

being explored to develop inhibitors of allergic diseases [16,18]. The activation of mast cells is initially dependent on the interaction of FcεRI with the Src kinases Lyn and Fyn and subsequently on the downstream activation of Syk and other tyrosine kinases [15]. However, Lyn also exhibits a negative role and this is the predominant feature of Lyn in early signaling events. In contrast,

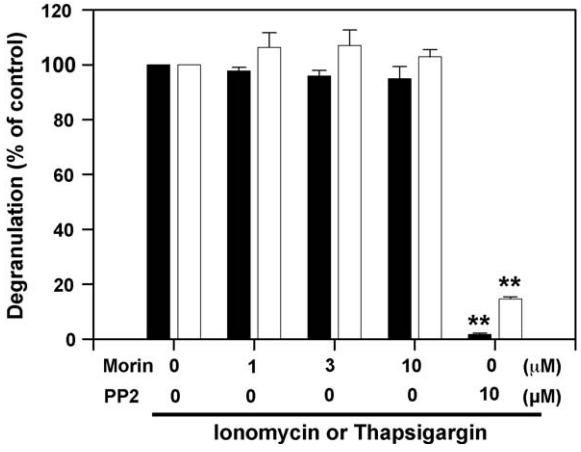

Fig. 5. Effect of morin on thapsigargin or ionomycin-stimulated degranulation in mast cells. The RBL-2H3 cells were stimulated with 300 nM thapsigargin (■) or 1 μM ionomycin (□), and degranulation was determined by measuring the activity of β-hexosaminidase in culture media. The values indicate mean ± S.E.M. from three independent experiments. PP2 is a general Src-family kinase inhibitor.

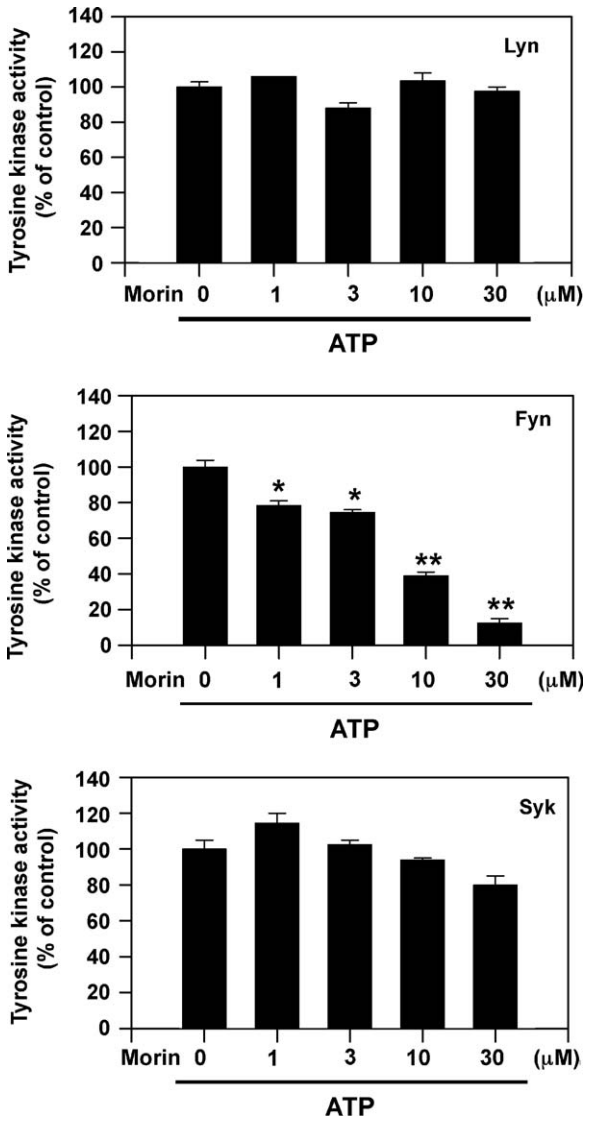

Fig. 6. Effect of morin on in vitro activity of Lyn, Fyn, or Syk. In a final reaction volume of 25 μl, each tyrosine kinase (Lyn, Fyn, or Syk) (5–10 mU) was incubated with 50 mM Tris, pH 7.5, 0.1 mM EGTA, 0.1 mM Na_3VO_4, 10 mM Mg Acetate and [γ-^{33}P-ATP] (specific activity approximately 500 cpm/pmol). The reaction buffers contained 250 μM Cdc2 peptide (KVEKIGEGTYGVVYK) as a Fyn substrate or 0.1 mg/ml poly(Glu, Tyr) 4:1 as a substrate for assay of Lyn and Syk, respectively. The reaction was initiated by the addition of the MgATP mixture. After incubation for 40 min at room temperature, the reaction was stopped by the addition of 5 μl of a 3% phosphoric acid solution. The reaction mixture (10 μl) was then spotted onto a Filtermat A and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting. The values indicate mean ± S.E.M. from three independent experiments.

Fyn is exclusively a positive regulator in mast cells [15,29]. Although it was originally reported that Fyn phosphorylates Gab2 leading to the activation of phosphatidylinositol 3-kinase, it has also been reported that Fyn activates Syk and downstream signals [30] as appears to be the case in our studies. Syk plays a critical role in mast cell activation, because it is responsible for the activation of several downstream signaling molecules, including LAT, Grb2-associated binder 2 (Gab2), and SLP-76 adaptor proteins and in this manner regulates the degranulation and production of inflammatory cytokines [15]. Therefore, the Src-family kinases and Syk are attractive targets for design of therapeutic inhibitors of mast cell-related allergic diseases. Currently, several compounds that inhibit the activation of mast cells at the level of receptors or intracellular signaling molecules, are under clinical trials [31,32].

In this study, morin significantly suppressed mast cell degranulation and was identified as a reversible inhibitor of Fyn kinase (Figs. 2C and 6). These effects likely account for the inhibitory activity of morin in mast cell degranulation [9,10]. Previous reports have revealed that morin suppresses intracellular calcium ion levels and protein kinase C theta phosphorylation in mast cells [9]. Protein kinase C and the calcium signal are signaling events that occur downstream of the Src-family kinases in IgE-mediated mast cells [15,33] and are essential for degranulation [22]. Syk is initially phosphorylated at tyrosine 317 by Src-family kinases, primarily Lyn, which is the first kinase to be activated by the aggregation of high affinity IgE receptors. We found that morin and a general Src-family kinase inhibitor PP2 potently inhibited the phosphorylation of Syk and LAT, a Syk downstream substrate (Fig. 4). It is of interest that morin specifically inhibited the positive regulator of masts cell Fyn kinase, not the negative regulator of mast cells Lyn kinase, in vitro (Fig. 6). Furthermore, morin did not suppress the activation of mast cells stimulated by thapsigargin and inonomycin (Fig. 5), indicating that its inhibitory effect is specific for the IgE-high affinity receptor-proximal signals. Although the possibility remains that morin may inhibit mast cell activation by other mechanisms, our results suggest that the primary effect of morin is the inhibition of Fyn kinase.

In addition to inhibiting the degranulation of mast cells, morin exhibits anti-inflammatory activity [3] and inhibits inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) expression in macrophages [34]. This anti-inflammatory activity of morin might act in synergy with its anti-allergic effect in the treatment of patients with allergic inflammation. Syk inhibition has previously been shown to reduce allergic airway inflammation [32,35,36]. Syk inhibitors are also being evaluated for use in nasal sprays for treating the symptoms of seasonal allergic rhinitis [19,37]. Morin is a potent inhibitor of Ag-stimulated degranulation and cytokine secretion in mast cells and of mast cell-mediated PCA in mice. The observations and known anti-inflammatory properties of morin, warrant further evaluation of its clinical utility in mast cell-mediated immediate and delayed allergic diseases.

Of note, we have reported that another phenolic aromatic compound, curcumin, also inhibits mast cell activation and the allergic response in vivo but does so by specifically inhibiting Syk in mast cells [20]. Collectively, these findings point to chemical structures that can favor inhibition of one FcεRI-related tyrosine kinase over another in mast cells. As both morin and curcumin are food products their toxicity would be expected to be minimal and they may provide a useful starting points in the design of specific inhibitors of tyrosine kinases that are recruited by FcεRI and other immunological receptors.

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