



Mycoepoxydiene inhibits antigen-stimulated activation of mast cells and suppresses IgE-mediated anaphylaxis in mice

Xiao-chun Xia^a, Qiang Chen^a, Kun Liu^a, Ping-li Mo^a, Jing-wei Zhu^a, Ming-qiang Zhuang^a, Yue-mao Shen^{b,*}, Chun-dong Yu^{a,**}

^a State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen, China

^b School of Pharmaceutical Sciences, Shandong University, Jinan, China

ARTICLE INFO

Article history:

Received 24 April 2013

Received in revised form 17 June 2013

Accepted 24 June 2013

Available online 13 July 2013

Keywords:

Mycoepoxydiene

Mast cell

Passive cutaneous anaphylaxis

IgE

Syk

ABSTRACT

Mycoepoxydiene (MED) is a polyketide isolated from a marine fungus associated with mangrove forest. It has been shown that MED has many kinds of effects such as anti-cancer and anti-inflammatory activities. However, its effects on anaphylaxis are still unknown. Mast cells play a pivotal role in IgE-mediated allergic response. Aggregation of the high affinity IgE receptor (FcεRI) on the surface of mast cell activates a cascade of signaling events leading to the degranulation and cytokine production in mast cells. Our study showed that MED could significantly suppress antigen-stimulated degranulation and cytokine production in mast cells and IgE-mediated passive cutaneous anaphylaxis (PCA) in mice. Furthermore, we found that MED suppressed antigen-induced activation of Syk, and subsequently inhibited the phosphorylation of PLCγ1, Akt, and MAPKs such as extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK), and p38 in mast cells. Collectively, our study demonstrates that MED can inhibit the activation of mast cells and protect mice from mast cell-mediated allergic response through inhibiting the activation of Syk. These results suggest that MED is a potential compound for developing a promising anti-anaphylaxis drug.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Anaphylaxis is a severe, systemic allergic reaction involving the respiratory and/or cardiovascular systems, usually with additional cutaneous and/or gastrointestinal features [1]. Anaphylaxis, especially food allergy, is a worldwide problem, with evidence of increasing prevalence in many countries. About 25% of people in developed countries suffer from allergy disorders which lead to heavy socio-economic burden [2].

Immunoglobulin E antibodies and mast cells have been convincingly linked to the pathophysiology of anaphylaxis [3]. Mast cell progenitors in the bone marrow can be induced by interleukin-3 (IL-3) to proliferate and differentiate into bone marrow-derived mast cells (BMMCs). Several kinds of receptors are expressed on the surface of BMMCs among which high affinity receptor for IgE (FcεRI) is the most well-known one [4,5]. Antigen-dependent activation of mast cells with specific IgE bound to their surface is the central event in anaphylaxis. The binding of antigen-specific IgE to FcεRI sensitizes mast cells and other effector cells to release mediators in response to subsequent encounter with specific antigens or crossreactive antigens [3]. Re-exposure to allergen

can trigger the cross-linking of FcεRI aggregation on the surface of mast cell [6,7]. Aggregation of FcεRI in turn activates Src family protein tyrosine kinases Lyn and Fyn [8–10]. This event leads to the stimulation of Syk through the phosphorylation of immune receptor tyrosine-based activation motif (ITAM) or through direct phosphorylation of Syk [8,11]. Syk activation can stimulate several pathways including phospholipase (PL)Cγ, PI3K/Akt, and mitogen-activated protein kinases (MAPKs), leading to mast cell degranulation and production of cytokines and lipid mediators [12,13]. Thus, mast cells exert a significant effect on allergic inflammation and provide a therapeutic target in the treatment of allergic diseases [2,14].

Mycoepoxydiene (MED) is a novel polyketide isolated from the marine fungus *Diaporthe* sp. (*D. sp.*) HLY-1 found in submerged rotten leaves of *Kandelia candel* in a mangrove forest in Fujian Province, China [15]. Previous studies have shown that MED had anti-microbial and anti-cancer activities [15,16]. Recently, we have demonstrated that MED could inhibit LPS-induced inflammatory responses and ovariectomy-induced osteoporosis in mice [17,18]. In macrophages, MED significantly inhibits LPS-induced expression of pro-inflammatory mediators such as tumor necrosis factor α (TNF-α), IL-1β, IL-6, and nitric oxide (NO) through blocking the activation of both NF-κB and MAPK pathways [19]. As MED displays an anti-inflammatory function, we wonder whether it can suppress allergic response which also shows the activation of NF-κB and MAPK pathways [8]. In this study, we demonstrated that MED could inhibit the degranulation and cytokine production in antigen-stimulated mast cells through blocking the activation of Syk.

* Correspondence to: Y. Shen, School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong 250012, China. Tel.: +86 531 88382108.

** Correspondence to: C. Yu, School of Life Sciences, Xiamen University, Xiang-An South Road, Xiamen, Fujian 361102, China. Tel.: +86 592 2182013; fax: +86 592 2182083.

E-mail addresses: yshen@sdu.edu.cn (Y. Shen), cdyu@xmu.edu.cn (C. Yu).

Table 1
Primers for real-time PCR.

Gene	Forward primers	Reverse primers
IL-6	AACGATGATGCACCTTGACAG	CTCTGAAGGACTCTGGCTTTG
TNF- α	ACGTGGAAGTGGCAGAAGAG	GGTCTGGCCATAGAACTGA
GAPDH	GACCACAGTCCATGCCATCAC	CATACCAGGAAATGAGCTTGAC

In addition, MED effectively alleviated antigen-induced passive cutaneous anaphylaxis (PCA) in mice.

2. Materials and methods

2.1. Materials

MED was isolated from the fermentation broth of *D. sp.* HLY-1 as described (Lin et al., 2005). The identity of MED was confirmed by HRMS and ^1H and ^{13}C NMR analysis, and the purity of MED exceeded 95.7% according to the HPLC analysis [19]. MED was dissolved in dimethylsulfoxide (DMSO) and stored at -20°C . RPMI 1640, DMSO, MTT, sodium pyruvate, Curcumin, antibody against β -actin and anti-DNP IgE (clone SPE-7) were obtained from Sigma Aldrich (Sigma, St Louis, MO, USA); DNP-human serum albumin (DNP-HSA) was purchased from Biosearch Technologies (Biosearch Technologies, Novato CA, USA); recombinant murine SCF and IL-3 were purchased from Peprotech Inc. (Peprotech, Rocky Hill, NJ, USA); anti-mouse CD117 (c-Kit)-PE, anti-mouse Fc ϵ RI-FITC, IL-6 ELISA kit, and TNF- α ELISA kit were obtained from eBioscience (eBioscience, San Diego, CA, USA); non-essential amino acid was purchased from Gibco (Gibco, Grand Island, NY, USA); 2-mercaptoethanol was obtained from AMRESCO (AMRESCO, Solon OH, USA); fetal bovine serum (FBS) was obtained from Hyclone (Thermo scientific, IL, USA); antibodies against phospho-p38, p38, phospho-JNK, JNK, phospho-ERK1/2, ERK1/2, phospho-Akt, Akt, PLC γ 1, and phospho-PLC γ 1 were purchased from Cell Signaling Technology (Danvers, MA, USA); antibody against Syk was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); antibody against phospho-Syk was purchased from ProSci (ProSci Incorporated, CA, USA).

Female BALB/c mice (6–8 weeks of age) were used for all experiments. Animals were maintained with specific pathogen free air at a temperature between 20 and 23 $^\circ\text{C}$ with 12-h light and dark cycles and a relative humidity of 50%. Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals. All animal experimental procedures were approved by Animal Care and Use Committee of Xiamen University (Protocol Number: XMULAC20120001). Every effort was made to reduce the suffering of animals.

2.2. Methods

2.2.1. Preparation of bone marrow mast cells (BMMCs)

BMMCs were cultured as described with modifications [20,21]. Bone marrow cells were obtained from mice and cultured in BMMC-complete medium comprising RPMI 1640, 10% FBS, 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 100 μM nonessential amino acids, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 10 ng/ml mouse IL-3, and 10 ng/ml mouse SCF. Nonadherent cells were transferred to fresh complete medium once a week. After 4–5 weeks, cell purity was determined by flow cytometric analysis for Fc ϵ RI and c-kit expression.

2.2.2. Cell viability assay

Cell viability was analyzed by MTT assay. A total of 6×10^3 cells were seeded in 96 well plates. After incubation of 24 h, 10 μl MTT (5 mg/ml) was added to each well. The plates were incubated for 4 h before addition of 100 μl lysis buffer (10% SDS in 0.01 M HCl). The absorbance was measured at 560 nm using a microplate reader.

2.2.3. β -Hexosaminidase (β -HEX) release assay

β -HEX release assay was performed as previously described with modifications [11,21,22]. BMMCs were deprived of SCF overnight, and then sensitized in complete RPMI 1640 with 1 $\mu\text{g}/\text{ml}$ anti-DNP IgE for 4 h. Cells were washed once in Tyrode's buffer (130 mM NaCl, 10 mM HEPES, 1 mM MgCl_2 , 5 mM KCl, 1.4 mM CaCl_2 , 5.6 mM glucose, and 1 mg/ml BSA, pH 7.4) and resuspended in Tyrode's buffer at 2×10^5 per well in 96 wells. Then, cells were pretreated with different concentrations of MED for 30 min and stimulated with DNP-HSA for 1 h. After induction, cells were harvested with 1% Triton X-100, and cell lysis or medium were mixed with *p*-nitrophenyl-*N*-acetyl- β -D-glucosamide (1 mM). The reaction was terminated by 100 μl of 0.2 M glycine (pH 10.7) after incubation for 1 h, and the OD was measured at a wavelength of 405 nm. Values are expressed as percentages of intracellular β -hexosaminidase released into the medium.

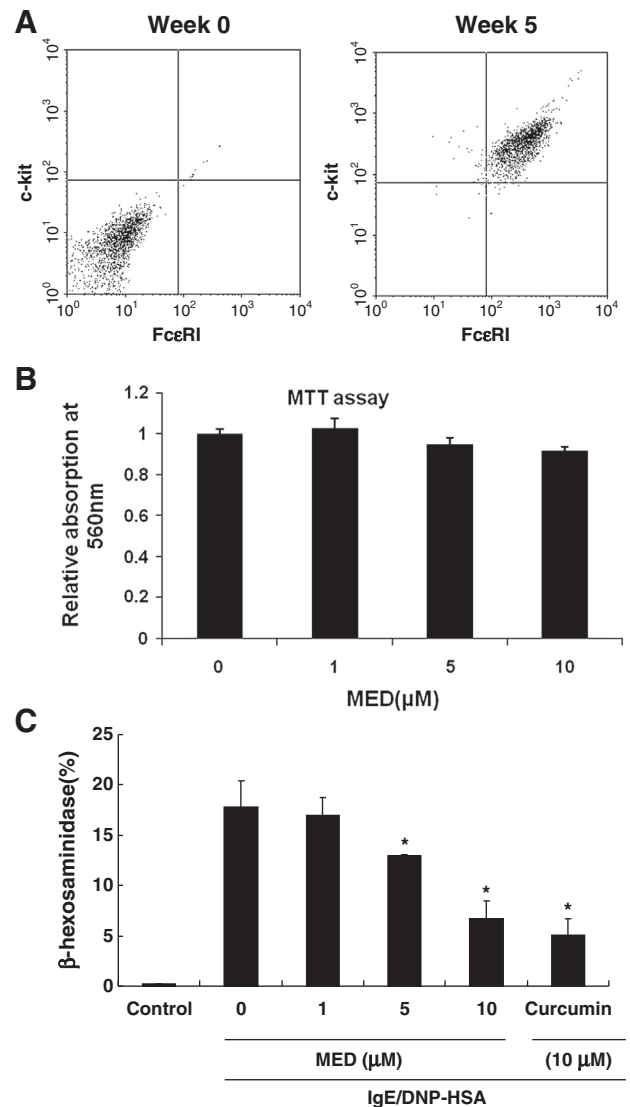


Fig. 1. MED inhibits the degranulation in antigen-stimulated mast cells. (A) Identification of BMMCs. Bone marrow cells were obtained from BALB/c mice and cultured in BMMC-complete medium. After 5 weeks, cells were identified by flow cytometric analysis for Fc ϵ RI and c-kit expression. The experiment was repeated for 3–5 times. Representative results are shown from three independent experiments. (B) BMMCs were seeded into 96-well plate at 6×10^3 cells/well. After adhesion, cells were treated with MED at indicated doses for 24 h. Cell viability was evaluated by MTT assay. (C) BMMCs were stimulated with IgE and DNP-HSA in the absence or presence of MED. Degranulation was measured by assessing β -hexosaminidase activity in the media or cell lysates. Values are shown as the mean $\pm$ SD from three independent experiments. * $p < 0.05$. Curcumin (10 μM) was used as a positive anti-anaphylaxis control agent.

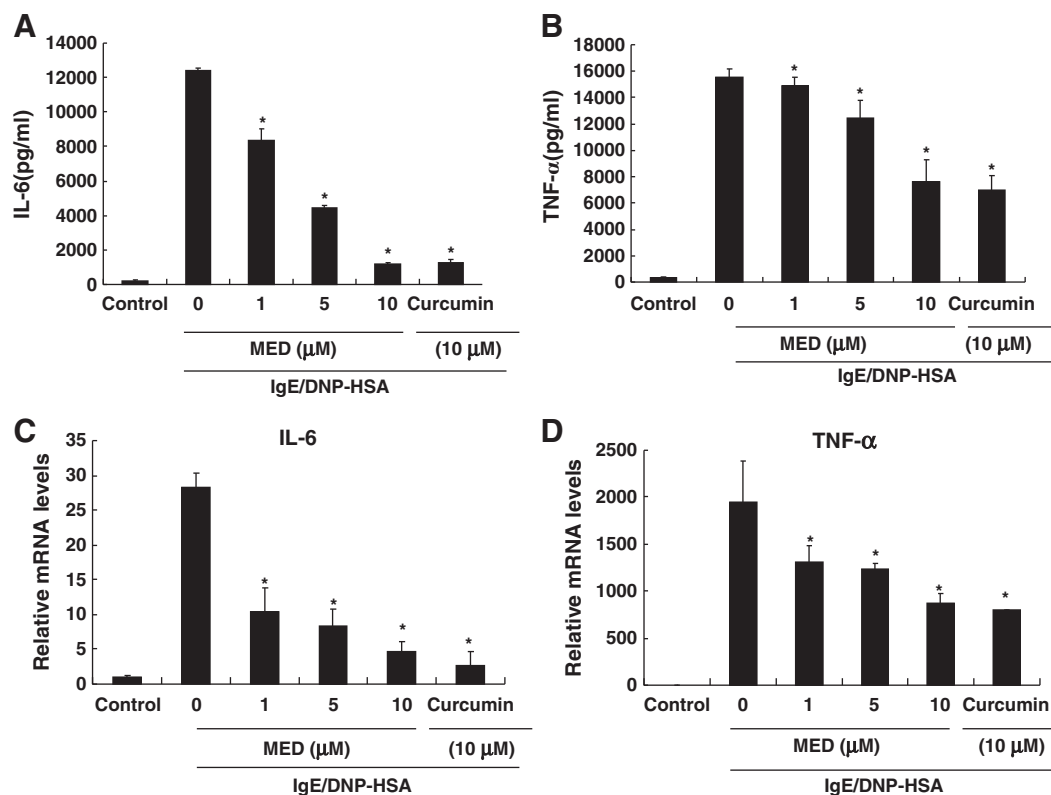


Fig. 2. MED inhibits the production of IL-6 and TNF- α in antigen-stimulated mast cells. BMMCs were pretreated with 1 μ g/ml anti-DNP IgE for 4 h and then were stimulated with DNP-HSA (100 ng/ml) in the absence or presence of MED overnight at 37 °C in 5% CO₂. (A and B) The amounts of IL-6 and TNF- α in the medium were measured using ELISA assay kits. (C and D) The mRNA levels of IL-6 and TNF- α were measured by real-time PCR. Values are shown as the mean $\pm$ SD from three independent experiments. * p < 0.05. Curcumin (10 μ M) was used as a positive anti-anaphylaxis agent.

2.2.4. Cytokine assay

Measurement of IL-6 and TNF- α production by BMMCs was performed as described with modifications [22]. BMMCs were deprived of SCF overnight, and then sensitized in complete RPMI 1640 with 1 μ g/ml anti-DNP IgE for 4 h. Cells were stimulated by DNP-HSA with or without different concentrations of MED overnight. The levels of IL-6 and TNF- α in medium were measured using ELISA kits according to the manufacturer's instructions.

2.2.5. Passive cutaneous anaphylaxis (PCA)

PCA was performed as previously described with modifications [7]. Mice were lightly anesthetized, the right ears were injected intradermally with 1 μ g anti-DNP IgE in 20 μ l PBS, and the left ears were injected with 20 μ l PBS as control. Twenty-four h later, mice were injected intravenously with 200 μ l of 1% Evan's blue dye containing 100 μ g DNP-HSA. For MED treatment, mice were pretreated (i.p. injection) with varying amounts of MED for 30 min before DNP-HSA injection. The mice were killed after injection with DNP-HSA, and the ears were removed and incubated in 1 ml formamide at 54 °C for 48 h. Absorbance of the resulting supernatants was measured at 610 nm. The relationship between Evan's blue concentration and absorbance was linear, indicating that the absorbance represented the quantity of Evans blue extravasation.

2.2.6. Quantitative real-time PCR

Total RNA was isolated with Trizol reagent (Invitrogen) according to the manufacturer's instructions. The cDNA was synthesized from 2 μ g of total RNA using MMLV transcriptase (ToYoBo, Shanghai, China) with random primers. Real-time PCRs were performed using SYBR Premix ExTaq (TaKaRa, Dalian, China). Quantification was normalized to the amount of endogenous GAPDH. Primers used for Real-time PCR are listed in Table 1.

2.2.7. Western-blot analysis

Cells were lysed with lysis buffer (200 mM Tris-HCl (pH 7.5), 1.5 M NaCl, 10 mM EDTA, 25 mM sodium pyrophosphate, 10 mM glycerolphosphate, 10 mM sodium orythovanadate, 50 mM NaF, 1 mM PMSF, in combination with protein inhibitor cocktail). Thirty micrograms of protein lysates of each sample was subjected to SDS-PAGE and transferred onto nitrocellulose membranes. Blots were incubated with the specific primary antibodies overnight at 4 °C. After being washed three times for 15 min each with TBST (TBS + 0.1% Tween20), blots were incubated with horseradish peroxidase-conjugated secondary antibody (Pierce, Rockford, IL, USA) and visualized by chemiluminescence. The band density was quantified by densitometry using Scion Image software and normalized to β -actin levels.

2.2.8. Statistical analysis

Data were collected from several independent experiments, with three replicates per experiment. All data were analyzed with one-way ANOVA with post-Tukey's post test in SPSS 11.0 and p < 0.05 was considered to be statistically significant. Bars in the graph represent standard deviation (S.D.).

3. Results

3.1. MED inhibits degranulation of antigen-stimulated BMMCs

Mature mast cells have specific surface markers such as Fc ϵ RI and c-kit. Therefore, bone marrow-derived mast cells (BMMCs) were identified by flow cytometric analysis for Fc ϵ RI and c-kit expression after incubation of bone marrow cells with BMMC-complete medium for 5 weeks. As shown in the right panel of Fig. 1A, after 5-week culture in BMMC-complete medium, 98.1% of bone marrow cells exhibited

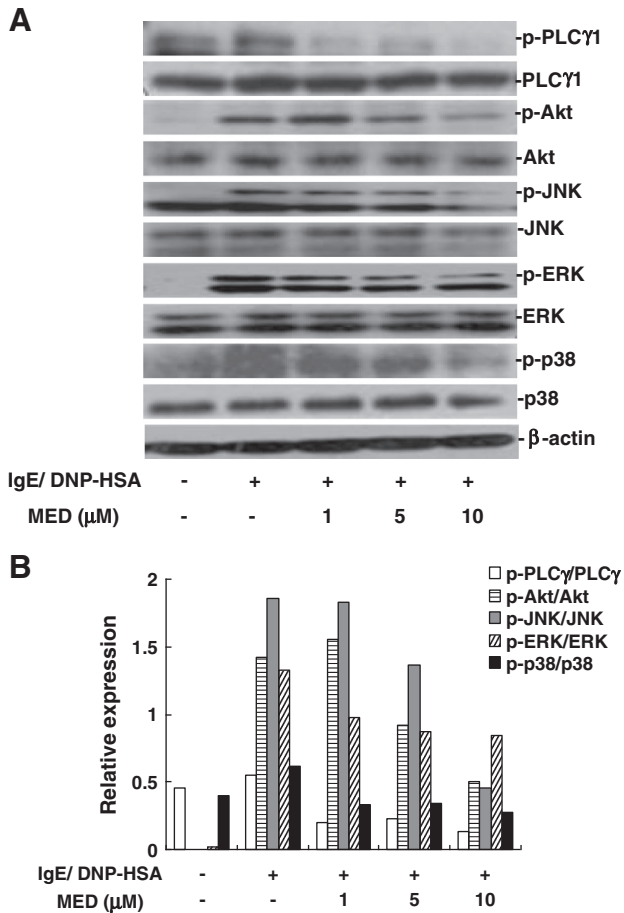


Fig. 3. MED inhibits the phosphorylation of PLC γ 1, Akt, and MAPKs in antigen stimulated mast cells. BMMCs were stimulated with anti-DNP IgE 1 μ g/ml for 4 h, and then treated with DNP-HSA for 5 min with or without MED pretreatment for 1 h. (A) Representative images are shown from three independent experiments. (B) Values are expressed as the ratios of phospho- versus non-phospho proteins.

increased expression of Fc ϵ RI and c-kit, indicating that bone marrow cells differentiated into mature mast cells.

Before examining the effect of MED on the allergic response of BMMCs, we measured the cytotoxicity of MED to BMMCs using MTT assay. As shown in Fig. 1B, MED has no apparent cytotoxicity to BMMCs in the concentration ranges from 1 to 10 μ M. Therefore, MED at concentrations up to 10 μ M was used in in vitro experiments.

IgE-mediated mast cell activation and allergic response show the features of degranulation and inflammatory mediator production [8]. Histamine is one of the most important mediators in allergic response. Mast cells release histamine during degranulation, and β -hexosaminidase is released along with histamine. Therefore, β -hexosaminidase is frequently used as a degranulation marker [23]. To investigate the effect of MED on antigen-induced mast cell degranulation, we measured β -hexosaminidase release from DNP-HSA antigen-stimulated BMMCs in the presence or absence of MED. Our results showed that mast cell degranulation induced by DNP-HSA was significantly inhibited by MED in a dose-dependent manner (Fig. 1C), indicating that MED can suppress the activation of mast cells.

3.2. MED suppresses cytokine production in antigen-stimulated BMMCs

Cytokines such as IL-6 and TNF- α are important for allergic inflammation mediated by mast cells [24,25]. To examine whether MED can affect the expression of IL-6 and TNF- α in antigen-stimulated mast

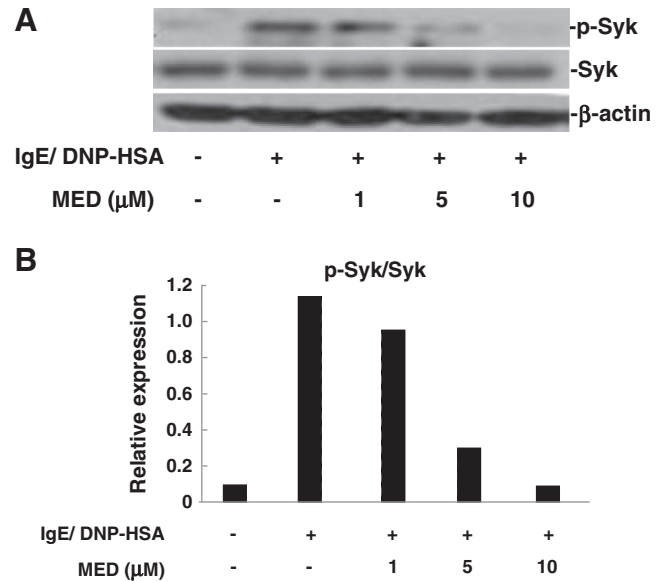


Fig. 4. MED inhibits Syk phosphorylation in antigen-stimulated mast cells. BMMCs were stimulated with anti-DNP IgE 1 μ g/ml for 4 h, and then treated with DNP-HSA for 5 min with or without MED pretreatment for 1 h. (A) Representative images are shown from three independent experiments. (B) Values are expressed as the ratios of phospho- versus non-phospho proteins.

cells, IL-6 and TNF- α were measured after DNP-HSA treatment in the presence or absence of MED. As shown in Fig. 2A and B, MED could significantly inhibit the production of IL-6 and TNF- α in a dose-dependent manner. Furthermore, MED could significantly suppress the mRNA levels of IL-6 and TNF- α (Fig. 2C and D), indicating that MED inhibits antigen-stimulated cytokines expression at the transcriptional level.

3.3. MED inhibits the activation of PLC γ 1, Akt, and MAPKs in antigen-stimulated BMMCs through blocking Syk phosphorylation

In mast cells, re-exposure to allergen triggers cross-linking of Fc ϵ RI, which activates Src family protein tyrosine kinases Lyn and Fyn. Lyn initially phosphorylates Syk and then induces the activation of Cy1, a critical enzyme for generation of calcium signal required for degranulation [26]. Since MED can inhibit mast cell degranulation, we examined whether MED has effect on the activation of PLC γ 1. As shown in Fig. 3, MED significantly inhibited the phosphorylation of PLC γ 1, suggesting that MED inhibits degranulation in antigen-stimulated mast cells at least in part through blunting PLC γ 1 activation.

In addition, activation of PI3K/Akt and MAPKs such as JNK, ERK1/2 and p38, are required for mast cell degranulation and cytokine production [26]. The results showed that MED effectively suppressed the phosphorylation of Akt, JNK, ERK1/2, and p38 in antigen-stimulated mast cells (Fig. 3). The inhibitory effect of 10 μ M MED on the activation of PLC γ 1, Akt, and MAPKs in antigen-stimulated BMMCs was similar to that of 10 μ M Curcumin (Fig. S1), an anti-anaphylaxis agent [27].

Given that PLC γ 1, PI3K/Akt, JNK, ERK1/2, and p38 are downstream signaling pathways of Syk, we examined whether MED inhibits the activation of PLC γ 1, Akt and MAPKs in antigen-stimulated mast cells through blocking the activation of Syk. As shown in Fig. 4, MED effectively inhibited the phosphorylation of Syk in antigen-stimulated mast cells, indicating that MED inhibits the activation of PLC γ 1, Akt, and MAPKs through suppressing Syk activation.

3.4. MED inhibits IgE-mediated passive cutaneous anaphylaxis (PCA)

To investigate whether MED has inhibitory effects on anaphylaxis in vivo, mice were injected intradermally with anti-DNP IgE followed

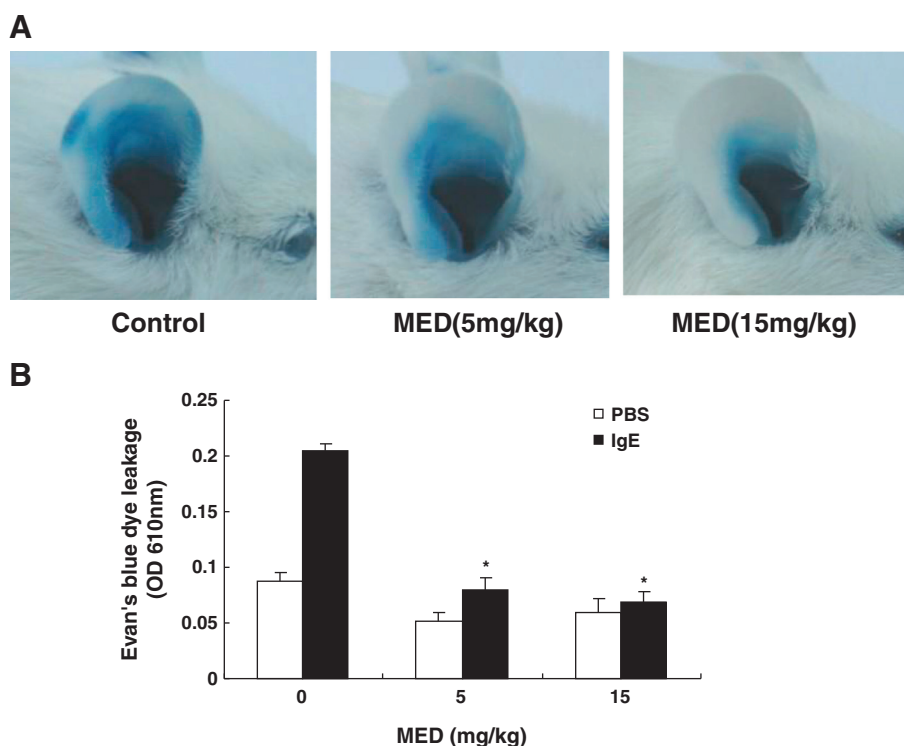


Fig. 5. MED effectively alleviates passive cutaneous anaphylaxis in mice. The right ears of mice were injected with 1 μ g anti-DNP IgE in 20 μ l PBS, and the left ears were injected with 20 μ l PBS as control. Twenty-four h later, mice were injected intravenously with 200 μ l of 1% Evan's blue dye containing 100 μ g DNP-HSA. Mice were pretreated with various amounts of MED for 30 min before DNP-HSA injection. (A) Representative images are shown. (B) Values are expressed as means $\pm$ SD from three independent experiments. * $p < 0.05$.

by intravenous injection of DNP-HSA plus Evan's blue dye with or without MED pretreatment for 30 min. The results showed that MED significantly suppressed IgE-mediated vascular permeability at the concentrations of 5 mg/kg and 15 mg/kg ($p < 0.05$) (Fig. 5). These results clearly demonstrated that MED was able to suppress IgE-mediated PCA reaction in vivo.

4. Discussion

Mast cells play a central role in both innate and adaptive immune responses. It has been demonstrated that allergic diseases including allergic asthma, rhinitis, atopic dermatitis, and food allergies are associated with mast cell activation [2]. The high affinity receptor for IgE (Fc ϵ RI) is critical for mast cell development and function [28]. Cross-linking of Fc ϵ RI by antigen results in mast cell degranulation, lipid mediator production, and cytokines production [7]. Traditional treatments of allergic diseases have some limitations such as lack of efficacy or severe side effects [29]. Thus, new remedies are being investigated for development of new drugs.

MED, a novel polyketide, was isolated from the marine fungus *D. sp. HLY-1* found in submerged rotten leaves of *K. cadel* in a mangrove forest [15]. It has been reported that MED has various kinds of biological effects such as anti-microbial, anti-cancer, and anti-inflammatory activities [15,16,19]. In our study, we found that MED not only markedly inhibited degranulation and cytokine production in antigen-stimulated BMMCs, but also suppressed antigen-induced passive cutaneous anaphylaxis in vivo, demonstrating that MED has an anti-anaphylaxis activity.

In antigen-stimulated mast cells, Src family kinases such as Lyn and Fyn are activated and subsequently phosphorylate the ITAM motif of Fc ϵ RI β and γ chains and Syk. Syk is a central signaling molecule that activates the downstream signaling molecules including PLC γ 1, PI3K/Akt, and MAPKs [30], which are important for mast

cells activation [31]. Activation of PLC γ 1 and PI3K/Akt results in PKC β activation and the increase of Ca $^{2+}$ influx, which are key regulators for mast cells degranulation [32]. MAPKs including ERK1/2, JNK and p38 are important for the cytokine production in mast cells [33]. In agreement with the notion that Syk plays essential roles in mast cell degranulation and cytokine production, it has been reported that Syk-deficient mast cells exhibit impaired degranulation and inflammatory cytokine production after Fc ϵ RI cross-linking [34]. Thus Syk becomes a prominent drug target for treating allergic disease. Nowadays many kinds of drugs are under investigating for inhibiting allergic response through suppression of Syk activation [35,36]. In this study, we found that MED can markedly inhibit the phosphorylation of Syk and subsequently suppress the phosphorylation of PLC γ 1, Akt, and MAPKs induced by DNP-HSA (Fig. 6). These results suggest that MED suppresses the degranulation and cytokine production at least in part through blocking the activation of Syk or upstream signaling (Fig. 6).

The PCA animal model is widely used to study IgE-mediated anaphylaxis in mice [11,20,36]. It is a localized cutaneous allergic reaction as a consequence of allergen-induced vascular permeability and plasma extravasation. Our study showed that MED significantly inhibited the PCA response in mice, implicating that MED may be a new drug for the effective treatment of IgE-mediated allergic diseases.

Collectively, our results demonstrate that MED, as a novel marine microbial compound, can suppress antigen-stimulated mast cells in vitro and protect mice from IgE-mediated anaphylaxis in vivo by inhibiting the activation of Syk in mast cells. Our findings not only elucidate that MED is a potential compound for the development of novel anti-anaphylaxis drugs, but also suggest that marine microbial bioactive compounds is an important source of new anti-anaphylaxis drugs.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.intimp.2013.06.029>.

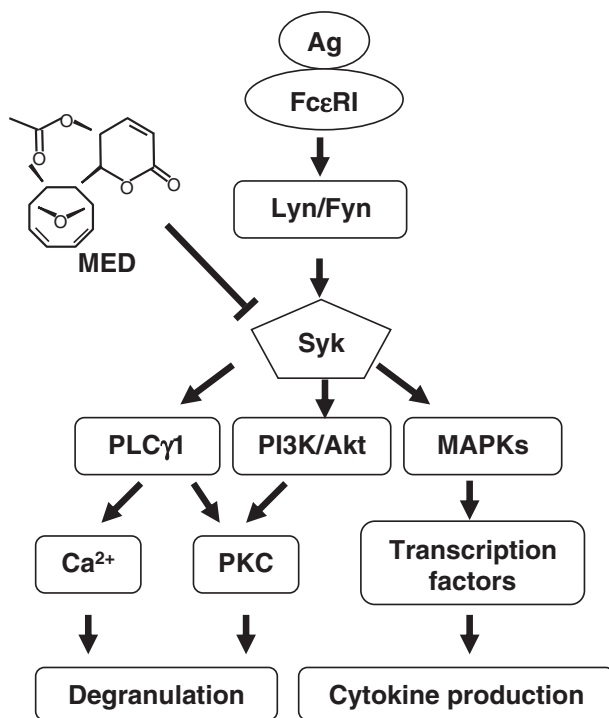


Fig. 6. Schematic representation of the mechanism by which MED inhibits the activation of mast cells. MED inhibits Syk phosphorylation and then inhibits the activation of PLC γ 1, PI3K/Akt, and MAPKs, which in turn leads to inhibition of degranulation and cytokine production.

Acknowledgments

This work was supported by grants from the Program for New Century Excellent Talents in University of the Ministry of Education (NCET-10-0718), the Fundamental Research Funds for the Central Universities (2012121038), and the Natural Science Foundation of China (31170819).

References

- [1] Laemmle-Ruff I, O'Hehir R, Ackland M, Tang ML. Anaphylaxis – identification, management and prevention. *Aust Fam Physician* 2013;42:38–42.
- [2] Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445–54.
- [3] Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;18:693–704.
- [4] Lu Y, Li Y, Jahng Y, Son JK, Chang HW. Citreoreosin inhibits degranulation and leukotriene C(4) generation through suppression of Syk pathway in mast cells. *Mol Cell Biochem* 2012;365:333–41.
- [5] Gilfillan GD, Straub T, de Wit E, Greil F, Lamm R, van Steensel B, et al. Chromosome-wide gene-specific targeting of the *Drosophila* dosage compensation complex. *Genes Dev* 2006;20:858–70.
- [6] Cheng C, Ng DS, Chan TK, Guan SP, Ho WE, Koh AH, et al. Anti-allergic action of anti-malarial drug artesunate in experimental mast cell-mediated anaphylactic models. *Allergy* 2012;68:195–203.
- [7] Hua X, Kovarova M, Chason KD, Nguyen M, Koller BH, Tilley SL. Enhanced mast cell activation in mice deficient in the A2b adenosine receptor. *J Exp Med* 2007;204:117–28.
- [8] Metcalfe DD, Peavy RD, Gilfillan AM. Mechanisms of mast cell signaling in anaphylaxis. *J Allergy Clin Immunol* 2009;124:639–46 [quiz 47–8].
- [9] Eiseman E, Bolen JB. Engagement of the high-affinity IgE receptor activates src protein-related tyrosine kinases. *Nature* 1992;355:78–80.
- [10] Parravicini V, Gadina M, Kovarova M, Odom S, Gonzalez-Espinosa C, Furumoto Y, et al. Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. *Nat Immunol* 2002;3:741–8.
- [11] Kim do K, Lee JH, Kim JW, Kim HS, Kim AR, Kim BK, et al. A novel imidazo[1,5-b]isoquinolinone derivative, U63A05, inhibits Syk activation in mast cells to suppress IgE-mediated anaphylaxis in mice. *J Pharmacol Sci* 2011;115:500–8.
- [12] Yang YJ, Chen W, Edgar A, Li B, Molkenstein JD, Berman JN, et al. Rcan1 negatively regulates Fc epsilonRI-mediated signaling and mast cell function. *J Exp Med* 2009;206:195–207.
- [13] Kawakami T, Galli SJ. Regulation of mast-cell and basophil function and survival by IgE. *Nat Rev Immunol* 2002;2:773–86.
- [14] Kinet JP. The essential role of mast cells in orchestrating inflammation. *Immunol Rev* 2007;217:5–7.
- [15] Lin X, Huang Y, Fang M, Wang J, Zheng Z, Su W. Cytotoxic and antimicrobial metabolites from marine lignicolous fungi, *Diaporthe* sp. *FEMS Microbiol Lett* 2005;251:53–8.
- [16] Wang J, Zhao B, Zhang W, Wu X, Wang R, Huang Y, et al. Mycoepoxydiene, a fungal polyketide, induces cell cycle arrest at the G2/M phase and apoptosis in HeLa cells. *Bioorg Med Chem Lett* 2010;20:7054–8.
- [17] Chen Q, Chen T, Li W, Zhang W, Zhu J, Li Y, et al. Mycoepoxydiene inhibits lipopolysaccharide-induced inflammatory responses through the suppression of TRAF6 polyubiquitination [corrected]. *PLoS One* 2012;7:e44890.
- [18] Zhu J, Chen Q, Xia X, Mo P, Shen Y, Yu C. Mycoepoxydiene suppresses RANKL-induced osteoclast differentiation and reduces ovariectomy-induced bone loss in mice. *Appl Microbiol Biotechnol* 2013;97:767–74.
- [19] Chen Q, Chen T, Li W, Zhang W, Zhu J, Li Y, et al. Mycoepoxydiene inhibits lipopolysaccharide-induced inflammatory responses through the of TRAF6 polyubiquitination. *PLoS One* 2012;7:e44890.
- [20] Taketomi Y, Sunaga K, Tanaka S, Nakamura M, Arata S, Okuda T, et al. Impaired mast cell maturation and degranulation and attenuated allergic responses in Ndr1g1-deficient mice. *J Immunol* 2007;178:7042–53.
- [21] Olenchok BA, Guo R, Silverman MA, Wu JN, Carpenter JH, Koretzky GA, et al. Impaired degranulation but enhanced cytokine production after Fc epsilonRI stimulation of diacylglycerol kinase zeta-deficient mast cells. *J Exp Med* 2006;203:1471–80.
- [22] Wu JN, Jordan MS, Silverman MA, Peterson EJ, Koretzky GA. Differential requirement for adapter proteins Src homology 2 domain-containing leukocyte phosphoprotein of 76 kDa and adhesion- and degranulation-promoting adapter protein in Fc epsilonRI signaling and mast cell function. *J Immunol* 2004;172:6768–74.
- [23] Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol* 2000;12:624–31.
- [24] Chai OH, Shon DH, Han EH, Kim HT, Song CH. Effects of Anemarrhena asphodeloides on IgE-mediated passive cutaneous anaphylaxis, compound 48/80-induced systemic anaphylaxis and mast cell activation. *Exp Toxicol Pathol* 2012;65:419–26.
- [25] Lee JH, Kim JW, Ko NY, Mun SH, Kim DK, Kim JD, et al. Camellia japonica suppresses immunoglobulin E-mediated allergic response by the inhibition of Syk kinase activation in mast cells. *Clin Exp Allergy* 2008;38:794–804.
- [26] Gilfillan AM, Tkaczuk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 2006;6:218–30.
- [27] Lee JH, Kim JW, Ko NY, Mun SH, Her E, Kim BK, et al. Curcumin, a constituent of curry, suppresses IgE-mediated allergic response and mast cell activation at the level of Syk. *J Allergy Clin Immunol* 2008;121:1225–31.
- [28] Kinet JP. The high-affinity IgE receptor (Fc epsilonRI): from physiology to pathology. *Annu Rev Immunol* 1999;17:931–72.
- [29] Holgate ST, Polosa R. Treatment strategies for allergy and asthma. *Nat Rev Immunol* 2008;8:218–30.
- [30] de Castro RO. Regulation and function of syk tyrosine kinase in mast cell signaling and beyond. *J Signal Transduct* 2011;2011:507291.
- [31] Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. *Immunol Rev* 2009;228:149–69.
- [32] El-Sibai M, Backer JM. Phospholipase C gamma negatively regulates Rac/Cdc42 activation in antigen-stimulated mast cells. *Eur J Immunol* 2007;37:261–70.
- [33] Lorentz A, Klopp I, Gebhardt T, Manns MP, Bischoff SC. Role of activator protein 1, nuclear factor-kappaB, and nuclear factor of activated T cells in IgE receptor-mediated cytokine expression in mature human mast cells. *J Allergy Clin Immunol* 2003;111:1062–8.
- [34] Costello PS, Turner M, Walters AE, Cunningham CN, Bauer PH, Downward J, et al. Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells. *Oncogene* 1996;13:2595–605.
- [35] Dong Kim J, Kim do K, Soon Kim H, Kim AR, Kim B, Her E, et al. Morus bombycis extract suppresses mast cell activation and IgE-mediated allergic reaction in mice. *J Ethnopharmacol* 2013;146:287–93.
- [36] Cheng C, Ng DS, Chan TK, Guan SP, Ho WE, Koh AH, et al. Anti-allergic action of anti-malarial drug artesunate in experimental mast cell-mediated anaphylactic models. *Allergy* 2013;68:195–203.