

Aspirin Augments IgE-Mediated Histamine Release from Human Peripheral Basophils via Syk Kinase Activation

Hiroaki Matsuo^{1,2}, Tomoharu Yokooji^{1,2}, Hironobu Morita¹, Mina Ooi¹, Kana Urata², Kaori Ishii³, Shunsuke Takahagi³, Yuhki Yanase³, Takaaki Hiragun³, Shoji Mihara³ and Michihiro Hide³

ABSTRACT

Background: Non-steroidal anti-inflammatory drugs (NSAIDs), especially aspirin, and food additives (FAs) may exacerbate allergic symptoms in patients with chronic idiopathic urticaria and food-dependent exercise-induced anaphylaxis (FDEIA). Augmentation of histamine release from human mast cells and basophils by those substances is speculated to be the cause of exacerbated allergic symptoms. We sought to investigate the mechanism of action of aspirin on IgE-mediated histamine release.

Methods: The effects of NSAIDs, FAs or cyclooxygenase (COX) inhibitors on histamine release from human basophils concentrated by gravity separation were evaluated.

Results: Benzoate and tartrazine, which have no COX inhibitory activity, augmented histamine release from basophils similar to aspirin. In contrast, ibuprofen, meloxicam, FR122047 and NS-398, which have COX inhibitory activity, did not affect histamine release. These results indicate that the augmentation of histamine release by aspirin is not due to COX inhibition. It was observed that aspirin augmented histamine release from human basophils only when specifically activated by anti-IgE antibodies, but not by A23187 or formyl-methionyl-leucyl-phenylalanine. When the IgE receptor signaling pathway was activated, aspirin increased the phosphorylation of Syk. Moreover, patients with chronic urticaria and FDEIA tended to be more sensitive to aspirin as regards the augmentation of histamine release, compared with healthy controls.

Conclusions: Aspirin enhanced histamine release from basophils via increased Syk kinase activation, and that the augmentation of histamine release by NSAIDs or FAs may be one possible cause of worsening symptoms in patients with chronic urticaria and FDEIA.

KEY WORDS

aspirin, basophils, food additives, histamine release, NSAID

INTRODUCTION

Aspirin, also known as acetylsalicylic acid, is widely used as an antipyretic, pain reliever, anti-inflammatory agent, and anticoagulant in a variety of diseases. It reduces the formation of prostaglandins and thromboxanes from arachidonic acid by inhibiting cyclooxygenases (COX)-1 and COX-2. COX-1 is responsible for the physiological production of prostagland-

ins whereas COX-2 is involved in the increased production of prostaglandins in inflammatory tissues.¹ Adverse effects such as gastrointestinal disorders often occur due to the inhibition of COX-1.²

Aspirin hypersensitivity, also known as aspirin intolerance, can be categorized in three types by their clinical presentations: i) aspirin-induced rhinitis and asthma or aspirin-exacerbated respiratory tract disease (AERD) of which the main symptoms are bron-

¹Department of Pathophysiology and Therapeutics, ³Department of Dermatology, Graduate School of Biomedical and Health Sciences, Hiroshima University and ²Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hiroshima University, Hiroshima, Japan.

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Correspondence: Hiroaki Matsuo, PhD, Department of Patho-

physiology and Therapeutics, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan.

Email: hmatsuo@hiroshima-u.ac.jp

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chospasm, rhinorrhea, conjunctivitis and/or flushing; ii) aspirin-induced urticaria and/or angioedema; and iii) a combination of i) and ii).³ Aspirin is also an aggravating factor in chronic idiopathic urticaria. Approximately 20 to 30% of patients with chronic urticaria react adversely to aspirin.⁴ The mechanism of aspirin hypersensitivity is not fully understood, but decreased prostaglandin (PG) E₂ levels due to COX-1 inhibition and increased production of cysteinyl leukotrienes (LTs), LTC₄, LTD₄, and LTE₄ are thought to be the underlying mechanisms of AERD pathogenesis.⁵ However, the pathogenesis of aspirin-induced urticaria/angioedema may be different from that of AERD. Decreased levels of PGE₂ and/or PGD₂, which decrease histamine release from mast cells, may be more important in the development of symptoms than an increase in LTs.⁶

Food-dependent exercise-induced anaphylaxis (FDEIA) is a particular form of food allergy. In FDEIA, allergic symptoms such as urticaria and anaphylaxis are induced by physical exercise after ingestion of specific food.^{7,8} It has been reported that aspirin may both augment the allergic symptoms and induce allergic reactions without exercise in patients with FDEIA.^{9,10} Previously, we demonstrated that aspirin increased the level of a blood antigen that correlated with clinical symptoms.^{11,12} This finding indicated that aspirin might facilitate allergen absorption through a gastrointestinal disorder. However, the administration of aspirin enhanced the reaction against an allergen in a prick test for patients with FDEIA, indicating that aspirin may also directly augment the degranulation of mast cells.¹³ Interestingly, the antigen-specific histamine release from antigen-induced peripheral basophils was augmented by aspirin in patients with pollen allergy.¹⁴ However, the cellular and molecular mechanisms of augmentation of histamine release by aspirin remain unclear. Moreover, food additives (FAs), such as tartrazine and benzoate, augment histamine release from basophils¹⁴ and may also trigger allergic symptoms similar to aspirin, although there is no clear evidence for this.¹⁵ The aim of this study is to evaluate the augmentation of histamine release by non-steroidal anti-inflammatory drugs (NSAIDs) and FAs and investigate its mechanism of action.

METHODS

REAGENTS AND MATERIALS

The following reagents were purchased: aspirin, mepirizole (1-(4-Methoxy-6-methyl-2-pyrimidinyl)-3-methyl-5-methoxy-1H-pyrazole), sunset yellow (5-[4-(Sodiooxysulfonyl)phenylazo]-6-hydroxynaphthalene-2-sulfonic acid sodium salt), Borudeaux S (1-(4-Sulfo-1-naphthylazo)-2-hydroxy-3,6-naphthalenedisulfonic acid trisodium salt), new coccine (2'-Hydroxy-[1,1'-azobisnaphthalene]-4,6',8'-trisulfonic acid trisodium salt) (Wako Pure Chemical Industries, Osaka, Japan);

A23187, formyl-methionyl-leucyl-phenylalanine (fMLP), thapsigargin, indomethacin, ibuprofen, sodium salicylate, benzoate, ethyl p-hydroxy benzoate, tartrazine, PGE₂ and PGD₂ (Nakalai Tesque Inc., Kyoto, Japan); diclofenac, ketoprofen, mefenamic acid, meloxicam, etodolac, and acetaminophen (Sigma-Aldrich, MO, USA); anti-phospho-Syk (Tyr 352) antibody (Cell Signaling Technology, MA, USA); anti-Syk antibody (Santa Cruz, CA, USA); FR122047 and SC-791 (Merck, Darmstadt, Germany); goat anti-human IgE antibodies (Bethyl Laboratories, Montgomery, TX, USA); HRP conjugated goat anti-rabbit IgG (Biosource, CA, USA).

HISTAMINE RELEASE ASSAY

This study was approved by the ethics committee at Hiroshima University. Sixteen chronic urticarial patients (3 males, 13 females, mean age: 43.3 years, range: 14-81), 11 patients with FDEIA (5 males, 6 females, mean age: 33.1 years, range: 8-55), and 10 healthy controls (5 males, 5 females, mean age: 22.3 years, range: 20-38) were enrolled. Venous blood was obtained from healthy adult volunteers who had given informed consent. Peripheral blood leukocytes containing approximately 1% basophils were prepared as described by Tanaka *et al.*,¹⁶ with slight modifications. Briefly, heparinized peripheral blood was mixed with 1% methylcellulose in saline and the solution was settled for 40 min at room temperature. After centrifugation of the leukocyte-rich upper layer at 450 *g* for 15 min, the cell pellets were washed twice in buffer A (137 mM NaCl, 5 mM glucose, 2.7 mM KCl, 0.4 mM NaH₂PO₄, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.03% human serum albumin). The intact leukocytes were then resuspended in 100 μ l buffer A containing 2 mM CaCl₂ and 1 mM MgCl₂. Once the crystalline compounds of NSAIDs, FAs and COX inhibitors were dissolved in methanol and air-dried in order to make them amorphous. The amorphous form of compounds were redissolved with buffer A containing 2 mM CaCl₂ and 1 mM MgCl₂ then this chemical solution and a stimulant such as goat anti-human IgE antibodies, A23187, fMLP or thapsigargin dissolved in the same buffer were added to the basophil suspension simultaneously. After incubation for 40 min at 37°C, the reactions were terminated by the addition of 800 μ l of ice-cold buffer A and centrifugation at 2000 *g* for 5 min at 4°C. The supernatants were moved to new tubes and the cell pellets were resuspended with 900 μ l of buffer A. Next, 100 μ l aliquots of 20% HClO₄ were added to the supernatants and cell suspensions. After centrifugation of these samples, the amounts of histamine were measured by HPLC using a Shim-pack VP-ODS column (4.6 \times 150 mm; Shimadzu, Kyoto, Japan). A 20 μ l aliquot of each sample solution was loaded onto the HPLC. Histamine was eluted with 100 mM sodium tartrate buffer (pH 4.4) containing 10

mM sodium 1-octane sulfonate and methanol (2.5 : 1 vol/vol) at a flow rate of 1.0 ml/min and a column temperature of 50°C. The column eluent was mixed with the derivatization reagent [400 mM sodium borate buffer (pH 9.2) and 10 mM o-phthalaldehyde in methanol (2 : 1 vol/vol)], which was delivered by a pump at a flow rate of 0.5 ml/min. The mixture was passed through a reaction coil at 50°C and the effluent was monitored fluorometrically (excitation: 360 nm; emission: 440 nm). Net histamine release was calculated as a percentage of the total amount of histamine in the cells after subtraction of spontaneous release.

BASOPHIL PURIFICATION

Ethylenediaminetetraacetic acid-treated whole blood was mixed with an equal amount of 2 mM EDTA in phosphate-buffered saline (PBS). The mixture was layered onto Ficoll-Paque PLUS (GE Healthcare, Buckinghamshire, UK) and centrifuged at 400 *g* for 30 min. The buffy coats were isolated and basophils were purified by means of magnetic bead sorting using a human basophil isolation kit II (MACS, Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's protocol.

DETECTION OF Syk PHOSPHORYLATION

Syk and phospho-Syk were detected with anti-Syk and anti-phospho-Syk antibodies. The basophils purified from 56 ml venous blood ($2.3\text{--}4.0 \times 10^5$ cells) were suspended in buffer A containing 2 mM CaCl₂ and 1 mM MgCl₂, and incubated with goat anti-human IgE antibodies in 200 µl per tube. After incubation for 10 min at 37°C, the reactions were stopped by adding ice-cold buffer A and centrifuged at 2000 *g* for 5 min at 4°C. The supernatants were moved to new tubes and the concentrations of histamine were measured. The cell pellets were immediately lysed in buffer for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and boiled. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore, MA, USA). The membrane was blocked with Tris-buffered saline pH 7.4 (TBS) containing 5% skimmed milk, and was incubated with or without 1 : 1000 diluted anti-phospho-Syk antibodies at 4°C overnight. The membrane was washed 3 times with TBS containing 1% Tween-20 (TBST), and incubated at room temperature for 1 h with 1 : 10,000 diluted HRP-labeled anti-rabbit IgG antibody (Biosource). The membrane was washed 3 times with TBST, and proteins bound to IgG were detected using an ECL-plus western blot detection reagent (GE Healthcare) and LAS-3000 imager (Fujifilm, Tokyo, Japan).

STATISTICAL ANALYSIS

Tukey's and Dunnett's test were used for statistical analysis. The statistical analyses were performed with

PRISM (GraphPad, CA, USA).

RESULTS

HISTAMINE RELEASE FROM HUMAN BASOPHILS IN THE PRESENCE OF ASPIRIN

We first evaluated the augmentation of histamine release from peripheral basophils treated with various concentrations of aspirin. The histamine release from basophils was enhanced most strongly by 1000 µM of aspirin and aspirin alone induced marginal histamine release even at high concentration (Fig. 1a). A bell-shaped anti-IgE dose-response of histamine release was observed (Fig. 1b). Aspirin (1000 µM) augmented the histamine release at all concentrations of anti-human IgE used. Aspirin inactivates COX by acting as an acetylating agent and an acetyl group binds covalently to a serine residue in the active site of COX. To ensure the inactivation of COX by aspirin, basophils were preincubated with 1000 µM aspirin for 30 min before stimulation with anti-IgE. A similar augmentation of histamine release was observed in this experiment, but spontaneous histamine release by preincubation with the buffer alone increased (Fig. 1c). Therefore, 42 ng/ml of anti-human IgE was used for stimulation of basophils, and basophils were not preincubated with tested compounds in subsequent experiments.

The effects of aspirin on histamine release from basophils were evaluated in 10 healthy subjects (Fig. 1d). Subject 8 was a non-responder to anti-IgE stimulus. Aspirin (1000 µM) significantly increased the release of histamine induced by anti-IgE antibodies from basophils of 7 of 9 responders. Basophils from the other two subjects, 7 and 10, showed an increasing tendency towards histamine release in the presence of 1000 µM aspirin. In contrast, 100 µM of aspirin did not show any apparent effects on histamine release from basophils of any subjects.

EFFECTS OF NSAIDs AND FAs ON HISTAMINE RELEASE

The effects of NSAIDs and FAs on histamine release by anti-IgE stimuli are shown in Figure 2. Because NSAIDs or FAs alone induced less than 5% of histamine release even at high concentration of 1000 µM, we calculated the percent of histamine release by subtraction the quantity of histamine released by chemical alone from that by anti-IgE with the chemical. Increased histamine secretion was observed in the presence of aspirin, mefenamic acid, diclofenac, etodolac, ketoprofen, benzoate and tartrazine. However, the effects of diclofenac and tartrazine were lost or decreased at a dose of 1000 µM, whereas indomethacin inhibited histamine release at 1000 µM in spite of the enhancement of histamine release at 10 µM. Ethyl p-hydroxy benzoate, Borudeaux S and sunset yellow also inhibited histamine release at a concentration of 1000 µM. Ibuprofen, meloxicam, mepiri-

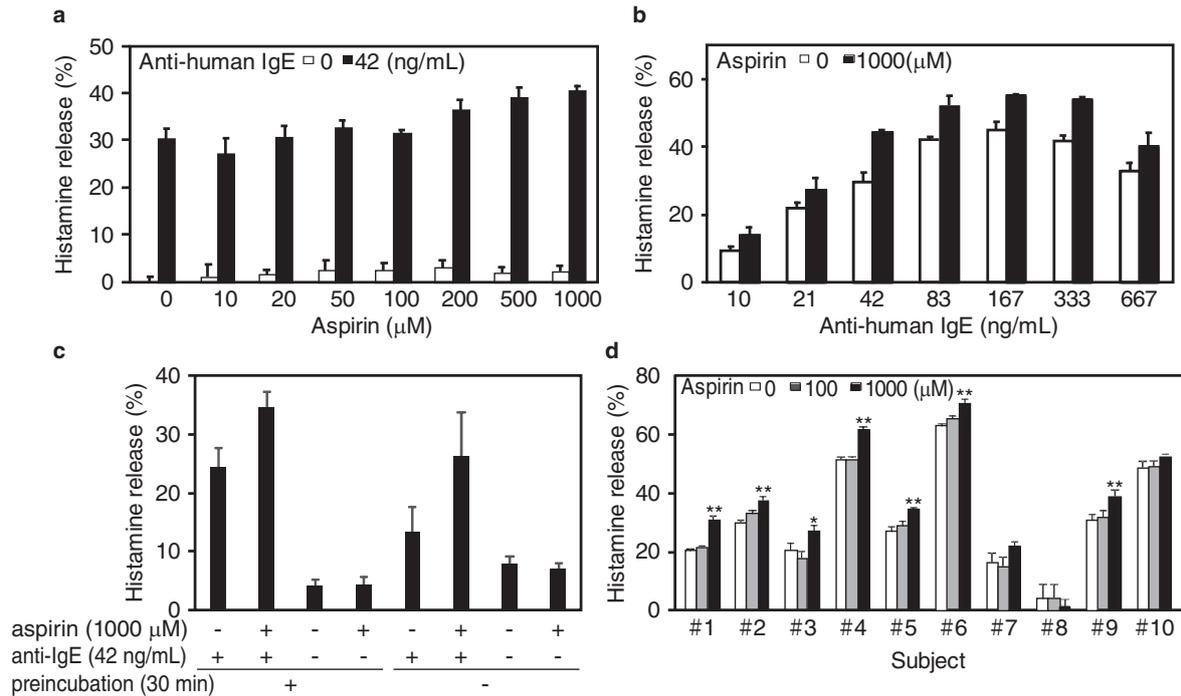


Fig. 1 The effect of aspirin on anti-human IgE induced histamine release from peripheral basophils. Dose-dependency of aspirin (**a**) and anti-human IgE (**b**) on histamine release from basophils of healthy subject #1 and the effect of preincubation with aspirin for 30 min before stimulation (**c**). The augmentation of histamine release by 100 and 1000 μM aspirin in 10 healthy subjects (**d**). Peripheral leukocytes were incubated with anti-human IgE antibodies in the presence or absence of aspirin for 40 min (**a**, **b**, **d**), or after basophils were preincubated with aspirin for 30 min anti-human IgE antibodies were added (**c**). * $P < 0.05$, ** $P < 0.01$ compared with controls.

zole, acetaminophen and new coccine had no effects on histamine release in the presence or absence of anti-IgE.

EFFECTS OF COX-1 AND COX-2 SELECTIVE INHIBITORS AND PGEs ON HISTAMINE RELEASE

The effects of FR122047 and SC-791, cell-permeable COX-1 and COX-2 selective inhibitors respectively, and PGE2 and PGD2, on histamine release induced by anti-IgE antibodies were examined. FR122047 (IC_{50} for human recombinant COX-1 and COX-2 is 28 nM and 65 μM , respectively)¹⁷ did not affect histamine release from basophils at any concentration tested. SC-791 did not affect histamine release at doses between 0.1 to 10 μM , but augmented at a dose of 100 μM (Fig. 3a, b). As the IC_{50} of SC-791 for COX-1 and COX-2 is 114 μM and 4 nM, respectively,¹⁸ the inhibition of COX was not related to the augmentation of histamine release. PGE2 and PGD2, derived from arachidonic acid by COX, are known to inhibit histamine release from human basophils, mouse bone marrow derived mast cells, and rat peritoneal mast cells.^{19,20} Histamine release from human basophils by anti-IgE was inhibited by PGE2 in a dose dependent manner in either the presence or absence of aspirin (Fig. 3c). On the contrary, PGD2 did not af-

fect histamine release (Fig. 3d). On the other hand, the augmentation of histamine release by aspirin was remained at any concentration of PGE2 or PGD2.

ASPIRIN INHIBITED HISTAMINE RELEASE INDUCED BY A23187, fMLP, AND THAPSIGARGIN

A23187, fMLP, and thapsigargin also induce histamine release from basophils and mast cells by different mechanisms from anti-IgE²¹: A23187 directly activates signaling pathways downstream of Ca^{2+} mobilization; fMLP activates a G protein-coupled receptor pathway; and thapsigargin induces Ca^{2+} influx by depletion of intracellular Ca^{2+} stores. In contrast to aspirin augmented IgE-mediated histamine release, 1000 μM of aspirin inhibited histamine release induced by A23187, fMLP, and thapsigargin (Fig. 4).

ASPIRIN ENHANCED SYK PHOSPHORYLATION

Syk tyrosine kinase plays a pivotal role in IgE-mediated histamine release from basophils. To analyze the phosphorylation of Syk and rule out the involvement of leukocytes other than basophils in the action of aspirin, we first confirmed that aspirin augmented the histamine release in purified basophils from three healthy donors (data not shown). Based on a report by Gibbs *et al.*²² that phosphorylation of

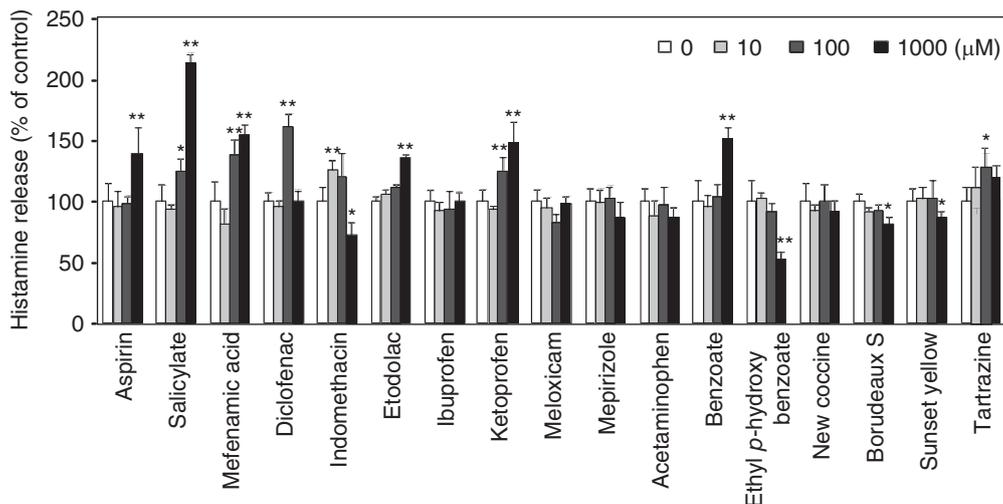


Fig. 2 Effects of NSAIDs on histamine release from human basophils. Peripheral leukocytes from subject 1 were incubated with anti-human IgE antibodies (42 ng/ml) and NSAIDs for 40 min. The percent values of histamine release were calculated by subtraction the quantity of histamine released by chemicals alone from that by chemicals and anti-IgE antibodies. The histamine release by chemicals alone at 1000 μ M were as follows: aspirin; 3.05 ± 0.74 (%), salicylate; 2.52 ± 0.59 , mefenamic acid; 2.57 ± 0.28 , diclofenac; 3.08 ± 0.41 , indomethacin; 0.89 ± 0.16 , etodolac; 1.88 ± 0.43 , ibuprofen; 2.54 ± 1.19 , ketoprofen; 1.82 ± 0.35 , meloxicam; 1.18 ± 0.20 , mepirizole; 0.97 ± 0.25 , acetaminophen; 2.64 ± 0.33 , benzoate; 2.36 ± 1.56 , ethyl *p*-hydroxy benzoate; 2.09 ± 0.22 , new cocaine; 2.93 ± 1.36 , bordeaux S; 0.57 ± 0.23 , sunset yellow; 1.47 ± 0.33 , tartrazine; 2.57 ± 2.34 . * $P < 0.05$, ** $P < 0.01$ compared with controls.

Syk is sustained for 10 minutes after stimulation by anti-human IgE antibodies, we examined the effect of aspirin on Syk phosphorylation in basophils stimulated by anti-IgE antibodies for 10 minutes in the presence or absence of 1000 μ M aspirin. The phosphorylation of Syk was determined by anti-phospho Syk Tyr 352 antibodies. The presence of 1000 μ M aspirin markedly increased Syk phosphorylation induced by anti-IgE antibodies (Fig. 5).

ASPIRIN SENSITIVITY OF IgE MEDIATED HISTAMINE RELEASE FROM BASOPHILS OF PATIENTS WITH CHRONIC URTICARIA AND FDEIA

Histamine release tests with anti-human IgE were conducted in 16 patients with chronic urticaria, 11 patients with FDEIA, and 10 healthy controls. Data from ten patients and one healthy control whose histamine release by anti-IgE were less than 10% (classed as non-responders) were excluded from the study. Addition of 1000 μ M aspirin augmented histamine release more than 10% in 14 of 17 patients (82.4%) and in 8 of 9 healthy controls (88.9%) (Fig. 6). A dose of 100 μ M aspirin augmented histamine release from basophils of seven patients and only one healthy control (11.1%).

DISCUSSION

In this study, we demonstrated that aspirin aug-

mented histamine release from human basophils and specifically activated the IgE receptor signaling pathway. The FAs, benzoate and tartrazine, also augmented histamine release from human basophils, suggesting that augmentation of histamine release by aspirin is not due to inhibition of COX. Moreover, our results indicate that patients with chronic urticarial and FDEIA tend to be more sensitive to aspirin in the augmentation of histamine release.

Wojnar *et al.*¹⁴ reported that aspirin (917 μ M), diclofenac (51 μ M), ketoprofen (375 μ M), indomethacin (0.1 μ M), mefenamic acid (35 μ M), salicylate (120 μ M), and tartrazine (1.7 mM) augmented histamine release by 25% in human basophils. Our data were almost in accordance with their results. They speculated that suppression of PG production by a number of drugs was one mechanism for the augmentation of histamine release as NSAIDs inhibit COX, a key enzyme for prostaglandin production. It has been shown that PGE₂ can reduce histamine release from human lung mast cells and basophils.²⁰ However, we found that neither ibuprofen nor FR122047, cell membrane permeable COX-1 selective inhibitors, affected histamine release. Moreover, benzoate and tartrazine, which have no COX inhibitory activity, enhanced histamine release. These results indicate that there is no correlation between inhibition of COX and an increase of basophil degranulation activity. The ad-

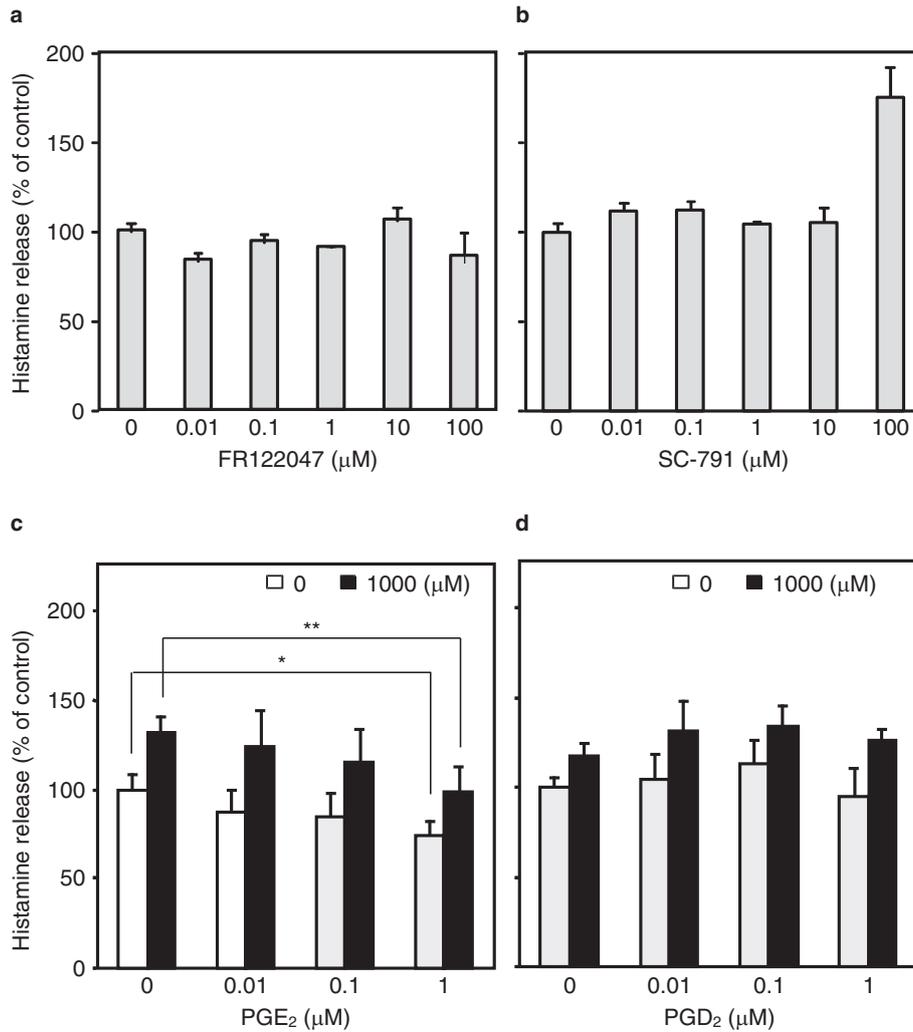


Fig. 3 Effect of cell-permeable COX-1 and -2 selective inhibitors (**a, b**) and PGs (**c, d**) on histamine release from basophils by anti-IgE antibodies. COX inhibitors, aspirin and PGs were co-incubated with anti-IgE and incubated for 40 min. * $P < 0.05$, ** $P < 0.01$.

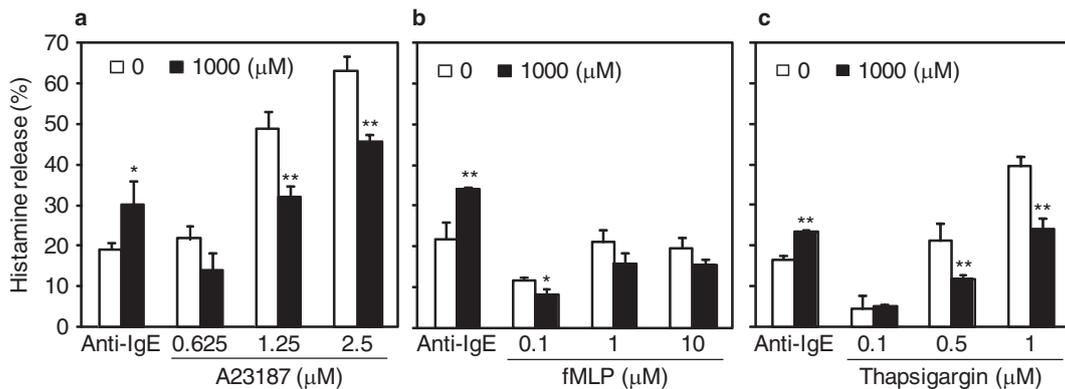


Fig. 4 Aspirin inhibits histamine release induced by A23187 (**a**), fMLP (**b**), and thapsigargin (**c**). Basophils from peripheral blood were incubated with anti-IgE (42 ng/ml), A23187, fMLP, or thapsigargin in the presence or absence of aspirin (control). * $P < 0.05$, ** $P < 0.01$ compared with control.

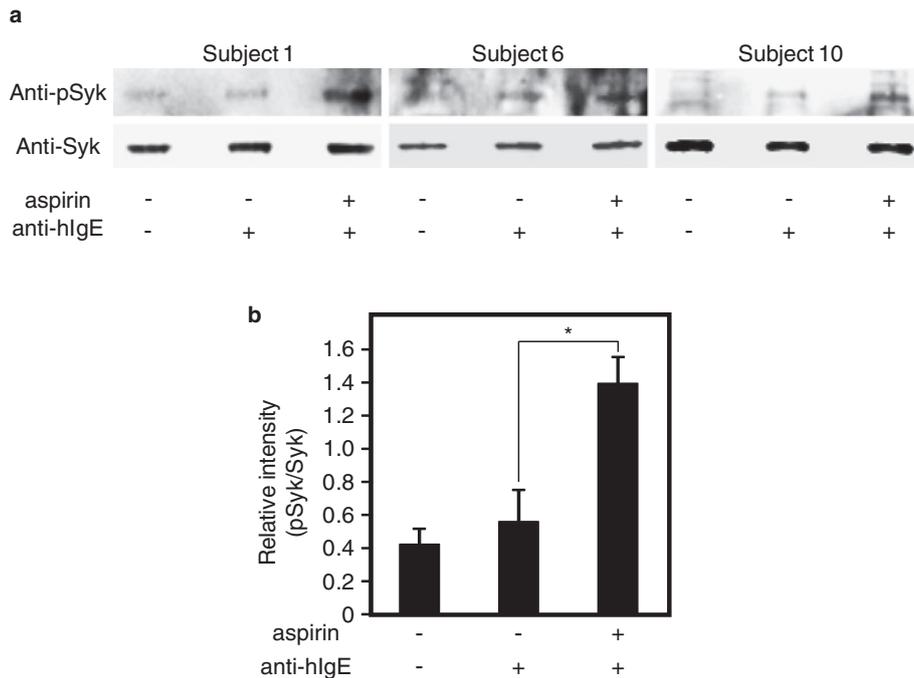


Fig. 5 Aspirin upregulates anti-IgE-stimulated phosphorylation of Syk kinase in purified basophils. Basophils purified from venous blood were incubated with anti-IgE in the presence or absence of aspirin for 10 min at 37°C. The reactions were stopped and the cell pellets were immediately lysed in buffer for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and boiled. Proteins were then transferred to a polyvinylidene difluoride membrane and phospho-Syk was detected by anti-phospho-Syk (Tyr 352) antibody. Reblotting with anti-Syk antibody verified equal protein loading between samples (**a**). Three experiments were carried out using basophils from three healthy subjects (one experiment in each donor). Bands in western blot were quantitated by image analysis and the mean relative intensity of the band was calculated (**b**). * $P < 0.05$.

dition of PGE₂ to the reaction mixture did not inhibit the enhancement of histamine release induced by aspirin (Fig. 3c). Therefore, there is likely another mechanism involved in the augmentation of histamine release by aspirin other than decreasing PG production due to COX inhibition.

Most NSAIDs and FAs that augmented the histamine release from basophils by anti-IgE antibodies in this study were acidic compounds. It seems likely that high concentration of acidic chemical enhanced histamine release. To eliminate that possibility we demonstrated that the acetic acid, one of the simplest carboxylic acids, has no effect on the augmentation of histamine release (data not shown). In addition to carboxylic acid residue the active chemicals have benzene ring in their structure indicating that those structures could be important to show the augmentation of histamine release.

The high affinity IgE receptor (FcεRI) is a tetrameric complex consisting of an α-chain, a β-chain, and two γ-chains. Binding of an allergen to two IgE molecules attached to the extra cellular domain of α-

chains, causes phosphorylation of Syk kinase and eventually diacylglycerol and inositol 1,4,5-trisphosphate (derived from phosphatidylinositol 4,5-bisphosphate by phospholipase Cγ) induce calcium influx into cells leading to degranulation of basophils. Intracellular FcεRI receptor signaling progresses through signaling and adaptor molecules such as Lyn, Syk, PI 3K, LAT and phospholipase Cγ.²³ Apart from anti-IgE antibodies, several substances can induce histamine release from human basophils, such as calcium ionophore (A23187), thapsigargin, and fMLP. Cell signaling cascades induced by these molecules overlap considerably with those of FcεRI. For example, fMLP induces histamine release by activation of PLC and PI 3K via formyl peptide receptor without Syk phosphorylation, which is a key reaction in the FcεRI pathway.²⁴⁻²⁶ Thapsigargin induced depletion of calcium in the endoplasmic reticulum and calcium influx through calcium release-activated calcium channels.²⁷ We observed no augmentation of histamine release by aspirin when basophils were activated with A 23218, thapsigargin, or fMLP. Moreover, aspirin aug-

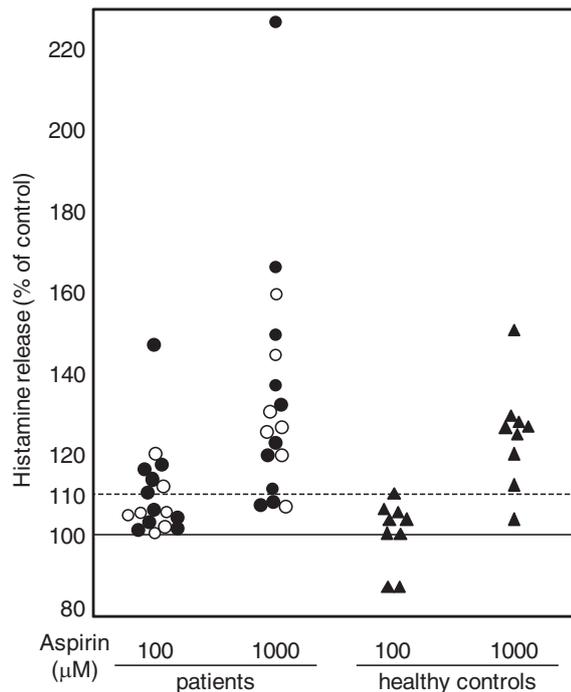


Fig. 6 Sensitivity to aspirin induced basophil histamine release in patients with chronic urticarial and FDEIA. Basophils obtained from peripheral blood were incubated with anti-IgE and aspirin concentrations of 100 or 1000 μM for 40 min at 37°C. Augmentation was expressed as a ratio of the net histamine release from basophils in the presence or absence of aspirin. Open circle- patients with chronic urticaria; closed circle- patients with FDEIA; closed triangle- healthy controls.

mented histamine release from crude leukocyte preparations and from purified human basophils, indicating that aspirin can directly influence basophils. Gibbs *et al.*²² reported that Syk phosphorylation in human basophils by anti-IgE stimulation lasted for 10 minutes after stimulation and decreased after 15 min. Based on this result, we analyzed Syk phosphorylation 10 minutes after stimulation in purified basophils and found that phosphorylation of Tyr352 in Syk was markedly increased by aspirin. These results suggest that aspirin acts upstream of Syk kinase in the Fc ϵ RI receptor signaling cascade. Aspirin inhibited histamine release induced by A23187, thapsigargin, and fMLP whereas it augmented histamine release by anti-IgE stimuli in human basophils, indicating that aspirin may also act as an inhibitor of the histamine release pathway following calcium influx.

Although histamine release from human basophils is augmented by aspirin, aspirin inhibits histamine release from rat RBL-2H3 cells, rat peritoneal mast cells,²⁸⁻³⁰ and mouse bone marrow derived mast cells.¹⁹ We confirmed that aspirin inhibits histamine-release-induced antigen in rat RBL-2H3 cells (data not shown). Siraganian *et al.*³¹ reported that following

PTEN phosphatase gene inhibition, degranulation in a MMC-1 mouse mast cell line was inhibited, but enhanced in bone marrow derived mast cells. This opposite effect may be explained by a subtle difference in the balance between PIP₂ and PIP₃ in the cell. The difference in the effects of aspirin between human basophils and rat RBL-2H3 or mast cells may be explained by the balance of signaling pathways including PIP₂ and PIP₃ in these cells.

In summary, aspirin enhances histamine release from human basophils via Sky kinase activation in vitro and the effect is specific to histamine release induced by Fc ϵ RI receptor stimulation. This may be a cause of the aggravation of urticaria by aspirin. In fact, the increased sensitivity to aspirin was seen in a certain population of patients and healthy subjects at a dose of 1000 μM , but more dominantly in patients at 100 μM (Fig. 6). Siebert DJ *et al.*³² reported that taking 600 mg of aspirin yielded peak plasma aspirin levels of 28 to 56 μM and peak salicylate, a main metabolite of aspirin, levels of 72 to 290 μM . Their results indicate that plasma concentration of aspirin cannot reach 100 μM at conventional aspirin dose. However, we speculated that local high concentration of aspirin in intestinal tract right after taking the medication, and salicylate that augments histamine release as well as aspirin can contribute to aggravation of urticaria in patients. On the other hand, it has been reported recently that exacerbation of allergic symptoms by aspirin in patients with FDEIA is not mediated by the direct effects of aspirin on mast cells or basophils.³³ Thus, further studies are required to clarify the relationship between the basophil or mast cell activation by aspirin and salicylate in vitro and the exacerbation of clinical symptoms.

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