Analysis of the Mechanism for the Development of Allergic Skin Inflammation and the Application for Its Treatment: Aspirin Modulation of IgE-Dependent Mast Cell Activation: Role of Aspirin-Induced Exacerbation of Immediate Allergy

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Abstract. Aspirin (acetylsalicylic acid) is a well-known nonsteroidal anti-inflammatory drug that can potentiate some acute allergies and causes adverse immunological reactions collectively referred to as aspirin intolerance, a disorder that induces urticaria, asthma, and anaphylaxis in response to oral administration of the drug. Aspirin also potentiates some acute allergies such as food-dependent exercise-induced anaphylaxis (FDEIA), a food allergy induced by physical exercise. The anti-inflammatory actions as well as the adverse immunological effects have been thought to be primarily due to inhibition of cyclooxygenase activity. However, a growing body of evidence suggests that mechanisms unrelated to inhibition of prostaglandin synthesis are involved. One key feature of aspirin intolerance is the overproductions of cysteinyl leukotrienes (LTs), in which mast cells have been implicated to play a role. In this review, we provide an overview of our current knowledge about the regulatory mechanisms of LTC4 secretion in mast cells and its modulation by aspirin, with a special emphasis on the role of Ca2+ signals. We also introduced our recent findings that mast cells express dihydropyridine-sensitive L-type Ca2+ channels (LTCCs) and that Ca2+ channels of this kind mediate aspirin modulation of LTC4 secretion in mast cells.

Keywords: mast cell, aspirin, L-type Ca2+ channel, leukotriene, allergic inflammation

Introduction

Aspirin (acetylsalicylic acid, ASA) is a well-known nonsteroidal anti-inflammatory drug (NSAID) that can potentiate some acute allergies and causes adverse immunological reactions collectively referred to as aspirin intolerance. ASA intolerance is a disorder that induces urticaria, asthma, and anaphylaxis in response to oral administration of the drug (1, 2). ASA also potentiates some acute allergies such as food-dependent exercise-induced anaphylaxis (FDEIA), a food allergy induced by physical exercise. Recently, ASA was shown to act as a powerful trigger of anaphylaxis in FDEIA patients (3). The anti-inflammatory actions of ASA have been attributed primarily to inhibition of prostaglandin synthesis (4). ASA acetylates Ser-530 of cyclooxygenase (COX) I and II, thereby blocking prostaglandin and thromboxane A2 synthesis, while therapeutic concentrations of ASA and salicylates (SA) inhibit COX II protein expression (5). An emerging view is that additional mechanisms, independent of COX inhibition, are involved in the anti-inflammatory actions of ASA and SA. These mechanisms include inhibiting the activity of the transcription factors nuclear factor κB (NF-κB) (6) and nuclear factor of activated T cells (NF-AT) (7), as well as inhibiting degranulation of basophils and mast cells (8).

One key feature of ASA intolerance is the overproduction of cysteinyl leukotrienes (cys-LTs) such as LTC4/D4/E4, which are all sequentially synthesized from arachidonic acid (AA). The cys-LTs are potent proinflammatory mediators and cause smooth muscle contraction and increased vascular permeability. Patients

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with ASA intolerance have significantly higher levels of cys-LTs in their bronchoalveolar lavage fluid and urine before and after oral ASA challenge (9).

Mast cells are important effector cells in allergic and inflammatory reactions and are the major producers of cys-LTs. Mast cells express the high-affinity IgE receptor (FcεRI) on their cell surface, and antigen cross-linking of FcεRI triggers biochemical cascades that lead to degranulation, cys-LTs and PGs secretion, and cytokine production. Moreover, mast cells are suggested to be responsible for cys-LT overproduction in ASA intolerance (10, 11). In addition, several studies have shown that an atopic background (high levels of serum IgE) is a risk factor for NSAID sensitivity (12).

**Dual effects of ASA and SA on IgE + Ag-mediated LTC₄ secretion in mast cells**

We analyzed the effects of ASA on IgE + antigen (Ag)-mediated LTC₄ secretion and on the signaling pathways involved in secretion in cultured mast cells including RBL-2H3 cells and murine bone marrow–derived mast cells (BMMCs) (13). Cells that had been passively sensitized with anti-TNP IgE were treated with ASA and then immediately stimulated with the Ag TNP–BSA (the basal LTC₄ level, 48 ± 11 pg/ml; IgE + Ag stimulation, 3601 ± 218 pg/ml, n = 5). Mortaz et al. (8) have reported that 10 mM ASA alone increases LT release, whereas ASA inhibits IgE + Ag-mediated β-hexosaminidase release. In both cell types, however, 10 mM ASA exhibited substantial cytotoxicity, and ASA up to 3 mM had a no effect on LT release. ASA had a dual effect on IgE + Ag-mediated LTC₄ secretion, depending on the concentration employed: at low concentrations (≤ 0.3 mM), ASA significantly enhanced LTC₄ secretion, while at high concentrations (>1 mM), it suppressed secretion (Fig. 1A). The optimal concentration of ASA was 0.1 – 0.3 mM in different experiments (approximately 1.5-fold greater secretion compared to IgE + Ag stimulation alone). Similar results were obtained when cells were exposed to the aspirin metabolite SA: at low concentrations (≤0.3 mM), SA significantly enhanced IgE + Ag-mediated LTC₄ secretion, while at high concentrations (>1 mM), it suppressed secretion (Fig. 1B). Essentially similar results were obtained with BMMCs (13). SA was approximately 3-fold more toxic than ASA. Treatment with ASA up to 3 mM for 4 h had a minimal effect on cell viability, as measured by phosphatidylserine externalization and propidium iodide permeability or trypan blue dye exclusion assay (data not shown). DMSO in 3 mM ASA (0.3%) had minimal effects on secretion and viability (97%). These results demonstrate that ASA and SA exhibit stimulatory effects on IgE + Ag-mediated LTC₄ secretion at non-toxic concentrations. Since ASA suppresses COX-1 and COX-2 activities and SA does not (14), these results suggest that the enhancement of LTC₄ secretion is not via suppression of COX activity.

**ASA enhances cPLA₂ activation independently of the ERK and p38MAPK pathways**

To gain insight into the mechanisms by which ASA modulates LTC₄ secretion in mast cells, we examined the
effect of ASA on the intracellular signaling pathways involved in LTC₄ synthesis, including activation of cytosolic phospholipase A₂ (cPLA₂), which mediates agonist-induced AA release in most cell types (15). The catalytic activity of cPLA₂ is phosphorylation-dependent. Phosphorylation of cPLA₂ on Ser-505 by ERK1/2 is necessary for cPLA₂-mediated AA release following stimulation of various cell types by many different agonists (16, 17). By using a monoclonal antibody that specifically recognizes the Ser-505 phosphorylation of cPLA₂, we found that IgE + Ag stimulation substantially induced cPLA₂ phosphorylation, and that low concentrations (≤0.3 mM) of ASA substantially (1.5-fold) enhanced the effect (Fig. 2A). Thus, ASA enhanced both of IgE + Ag-mediated LTC₄ secretion and Ser-505 phosphorylation of cPLA₂. We next examined whether ASA stimulated activation of ERK1/2. ERK1/2 is activated following dual phosphorylation of a threonine and a tyrosine residue by the upstream kinase MEK1/2. RBL-2H3 cells were treated with various concentrations (0.1 – 3 mM) of ASA prior to IgE + Ag stimulation, and activation of ERK1/2 was then assessed using a monoclonal antibody that specifically recognizes the dually phosphorylated (active) ERK1/2 (Thr202/Tyr204) (18). The phosphorylation of p42/44 ERK1/2 clearly increased following IgE + Ag stimulation (Fig. 2B). However, ASA treatment dose-dependently reduced the activation of ERK1/2 (Fig. 2B). The cPLA₂ Ser-727 residue is another site important for activation of the enzyme and is phosphorylated by MNK1-related protein kinases, which are activated by p38MAPK (19). We therefore examined the effect of ASA on p38MAPK activation. Western blot analysis using a monoclonal antibody recognizing the active enzyme, phospho-p38MAPK (Thr180/Tyr182), showed that IgE + Ag stimulation induced p38MAPK activation, but ASA at concentrations ranging from 0.1 to 3 mM did not substantially affect the phosphorylation level (Fig. 2C).

To determine the involvement of these MAPKs in LTC₄ secretion in our cell system, the effect of pharmacological inhibitors of selective MAPK pathways on IgE + Ag-mediated LTC₄ secretion was examined. U0126 (20 μM) and SB203580 (30 μM), which are selective inhibitors of ERK1/2 and p38MAPK, respectively, substantially (up to 50% inhibition) inhibited IgE + Ag-mediated LTC₄ secretion in RBL-2H3 cells, indicating that both MAPK pathways are involved in LTC₄ synthesis in our cell system. Collectively, our data indicate that ASA enhances cPLA₂ activation independently of the ERK and p38MAPK pathways.

**IgE + Ag-mediated LTC₄ secretion is Ca²⁺-dependent and ASA has a dual effect on Ca²⁺ responses**

Another key physiological regulator of LTC₄ synthesis is Ca²⁺. cPLA₂ is regulated by an increase in intracellular Ca²⁺, which binds to the amino-terminal C2 domain and induces cPLA₂ translocation to the nuclear envelope and the endoplasmic reticulum (20). Ca²⁺ is an important regulator of 5-lipoxygenase (5-LOX) as well, which catalyzes the addition of molecular oxygen to AA. Because Ca²⁺ entry from the extracellular space is a major source of increased [Ca²⁺]ᵢ, we determined whether LTC₄ secretion in mast cells was dependent on Ca²⁺ influx. As shown in Fig. 3A, EGTA chelation of extracellular Ca²⁺ completely abolished IgE-mediated LTC₄ secretion, indicating that this response is strictly dependent on Ca²⁺ influx. We then examined the effect of ASA on Ca²⁺ mobilization. ASA alone at concentrations ranging from 0.1 to 3 mM caused a minimal
increase in \([\text{Ca}^{2+}]\) (data not shown). However, ASA had a dual effect on IgE + Ag-mediated \([\text{Ca}^{2+}]\) increase: at low concentrations (\(\leq 0.3\) mM), ASA enhanced the response, while at high concentrations (>1 mM), it suppressed the \([\text{Ca}^{2+}]\) increase (Fig. 3B). Next, the effect of ASA on \([\text{Ca}^{2+}]\) mobilization from intracellular stores was examined in cells placed in nominally \([\text{Ca}^{2+}]\)-free medium. As shown in Fig. 3C, ASA dose-dependently reduced the effect of IgE + Ag stimulation. ASA reduced the maximal level of \([\text{Ca}^{2+}]\) with a minimum effective dose of 0.1 mM and caused an increase in the time to reach peak \([\text{Ca}^{2+}]\) levels (for ASA concentrations of 0, 0.3, 1, and 3 mM, the times to peak \([\text{Ca}^{2+}]\) levels were 30, 35, 50, and 60 s, respectively). In nonexcitable cells including mast cells, store-operated \([\text{Ca}^{2+}]\) entry (SOCE) is a main pathway of \([\text{Ca}^{2+}]\) influx (21). In RBL cells, the CRAC channels, the best known store-operated \([\text{Ca}^{2+}]\) channels (SOCs), are activated by a pharmacological emptying of intracellular \([\text{Ca}^{2+}]\) stores with thapsigargin (Tg) or by IgE + Ag stimulation (22, 23). We therefore examined whether ASA affected \([\text{Ca}^{2+}]\) influx through SOCs. Cells were treated with Tg for 10 min in nominally \([\text{Ca}^{2+}]\)-free medium in order to deplete intracellular \([\text{Ca}^{2+}]\) stores and activate SOCE. The cells were then incubated with ASA at the indicated concentrations or 10 \(\mu\)M 2-APB prior to the addition of 2 mM \([\text{Ca}^{2+}]\) (indicated by the arrow). The data are representative of five independent experiments. Modified with permission from Clin Immunol (Ref. 13).
almost completely abolished by 2-APB, a SOCE antagonist, indicating that the influx is SOC mediated. ASA (≥0.3 mM) suppressed Ca\(^{2+}\) influx in a dose-dependent manner. These results suggest that ASA may stimulate some other Ca\(^{2+}\)-entry pathway.

ASA enhances LTC\(_4\) secretion through facilitating LTCCs

Recent work has demonstrated that in various immune cells including T and B cells, in addition to SOCE, Ag-receptor stimulation can activate Ca\(^{2+}\) entry independently of Ca\(^{2+}\) store emptying. Moreover, we previously reported that IgE + Ag stimulation induces substantial Ca\(^{2+}\) entry even when SOCE is inactive. In addition, this Ca\(^{2+}\) entry was blocked by LTCC antagonists, but minimally affected by SOCE antagonists (24), suggesting the occurrence of LTCCs. More recently, we detected the expression of the LTCC \(\alpha_{1c}\) (Ca\(_{1.2}\)) transcripts and protein on mast cell surfaces. Inhibition of the LTCC activity by pharmacological antagonists such as nifedipine and diltiazem as well as small interfering RNA knockdown of \(\alpha_{1c}\) (Ca\(_{1.2}\)) LTCCs blocks IgE + Ag-mediated interleukin-13 and tumor necrosis factor-\(\alpha\) productions, but not degranulation (25). These findings suggest that mast cells express functional LTCCs, which are activated to regulate mediator secretion upon IgE + Ag stimulation. Therefore, we examined whether ASA might target this kind of Ca\(^{2+}\) channels. Consistent with our previous studies (24, 25), IgE + Ag stimulation increased [Ca\(^{2+}\)], in Ca\(^{2+}\) store-depleted cells. This effect was absent in Ca\(^{2+}\)-free conditions,
indicating that it was due to entry of extracellular Ca\textsuperscript{2+} (Fig. 4A). This Ca\textsuperscript{2+} entry was substantially reduced by the LTCC antagonist nifedipine but minimally affected by 2-APB. On the other hand, 2-APB completely abolished Ca\textsuperscript{2+} influx through SOCs, while nifedipine enhanced it (Fig. 4: B and C). ASA had a dual effect on this LTCC channel activity: at low concentrations (≤0.3 mM), ASA enhanced it, while at high concentrations (>1 mM), suppressed it (Fig. 4D). These results demonstrate that ASA enhances DHP-sensitive LTCCs.

Next, we determined whether the DHP-sensitive LTCCs were involved in LTC\textsubscript{4} secretion. Tg could induce substantial LTC\textsubscript{4} secretion under normal conditions: 1 μM Tg was more effective than IgE + Ag stimulation with the optimal Ag concentration (cell), ASA enhanced it, while at high concentrations (>1 mM), suppressed it (Fig. 4D). These results demonstrate that ASA enhances DHP-sensitive LTCCs.

Concluding remarks

It has been demonstrated that Ca\textsuperscript{2+} influx through CRAC channels regulates ERK activation, thereby controlling cPLA\textsubscript{2} activation and LTC\textsubscript{4} secretion (22, 23). We demonstrated here the occurrence of another Ca\textsuperscript{2+}-dependent pathway that leads to cPLA\textsubscript{2} activation and LTC\textsubscript{4} secretion independently of ERK activation. This pathway is mediated by DHP-sensitive LTCCs, which are pharmacologically distinct from SOCs (Fig. 6). Furthermore, it was demonstrated that ASA modulates LTC\textsubscript{4} secretion through this LTCC-mediated pathway. Although the biological significance of these findings remained to be elucidated, it is noteworthy that the LTCC-mediated LTC\textsubscript{4} secretion is enhanced by mitochondrial depolarization, while the SOC-mediated LTC\textsubscript{4} secretion is blocked (13). In the inflammatory milieu, mast cells may be exposed to oxidative stress, the major cause of mitochondrial depolarization, leading to inactivation of the SOC-mediated LTC\textsubscript{4} secretion. However, in the presence of ASA (especially at low doses), mast cells may produce substantially LTC\textsubscript{4} secretion through the LTCC pathway, leading to the exacerbation of IgE-mediated allergic reactions. Better understanding of the molecular mechanisms underlying ASA/SA modulation of LTCCs could lead to a new approach for the treatment of ASA-induced allergic and inflammatory disorders such as ASA intolerance and FDEIA.
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