

Pathogenesis of Aspirin-Induced Reactions in Aspirin-Exacerbated Respiratory Disease



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KEYWORDS

- Aspirin-exacerbated respiratory disease • Pathogenesis • Cysteinyl leukotrienes
- Prostaglandins • Mast cell • Platelet-leukocyte aggregates

KEY POINTS

- Aspirin-induced reactions in aspirin-exacerbated respiratory disease (AERD) result from the inhibition of COX-1 and cause a spectrum of reaction symptoms that range from localized respiratory symptoms to systemic symptoms involving the gastrointestinal tract and skin.
- The route of aspirin exposure and premedication with leukotriene modifying drugs (LTMDs) (ie, montelukast, zafirlukast or zileuton) influence the severity of aspirin-induced reactions. Intranasal desensitization protocols and the use of LTMDs allow for decreased bronchospasm and lowered rates of extrarespiratory symptoms.
- The release of tryptase, cysteinyl leukotrienes, histamine, and prostaglandin D₂ supports a central role for the mast cell in aspirin-induced reactions.
- Platelet-leukocyte aggregates are increased in AERD and are hypothesized to contribute to the overproduction of cysteinyl leukotrienes.
- Future studies of therapeutics that target mast cells, eosinophils, platelets, and inflammatory lipid and innate type 2 immune mediators in carefully phenotyped subjects are the key to improving our understanding of the classic aspirin-induced reactions in AERD.

INTRODUCTION

Aspirin-exacerbated respiratory disease (AERD) encompasses 2 distinct disease phases: the chronic baseline inflammation of the upper and lower respiratory tract characterized by nasal polyposis and asthma and the acute hypersensitivity reactions triggered by inhibitors of cyclooxygenase (COX)-1. As Drs Stevenson and Szczeklik¹

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nicely observed, “exposure to aspirin [or other COX-1 inhibitors] does not initiate or even perpetuate the underlying respiratory inflammatory disease”¹; yet the acutely exaggerated pathophysiology observed in the setting of an aspirin-induced respiratory reaction not only serves as the diagnostic gold standard but also offers insight into the cellular and biochemical derangements that underlie AERD. Our current understanding of the pathogenesis of aspirin reactions in AERD comes from studies of aspirin challenges performed in carefully phenotyped subjects. Our future progress in this disease lies in interventional trials of targeted therapeutics in the same carefully phenotyped populations.

CLINICAL FEATURES OF REACTIONS

The consistent clinical features of aspirin-induced reactions have greatly informed our current understanding of the pathophysiology of AERD. Classically, exposure to aspirin elicits upper and/or lower respiratory symptoms within 30 to 180 minutes in patients with AERD. In addition to aspirin, these respiratory reactions are triggered by any inhibitor of COX-1 (eg, ibuprofen, naproxen, ketorolac) and can even be triggered by high doses of acetaminophen (≥ 650 mg), which has mild COX-1 inhibitor properties,² but not by selective COX-2 inhibitors.^{3,4} These reactions represent hypersensitivity reactions, which are not immunoglobulin (Ig) E-dependent and, therefore, are not formally classified as allergic.

In North America there are 2 well-validated and most often clinically applied aspirin challenge protocols,^{5,6} involving oral aspirin and/or intranasal instillation of ketorolac, with a third protocol in Europe⁷ that uses intranasal lysine aspirin. In addition to respiratory symptoms, a subset of patients with AERD also develops extrarespiratory or systemic symptoms involving the skin and gastrointestinal tract following exposure to a COX-1 inhibitor.^{6,8} The route of aspirin challenge and the premedication regimen selected are the key predictors of both respiratory symptom severity and rates of extrarespiratory symptoms. In a study of 677 patients undergoing an oral aspirin challenge protocol, 38.6% of patients who were not on a leukotriene-modifying drug (LTMD) ($n = 417$) had a decrease in forced expiratory volume in the first second of expiration (FEV_1) of greater than 20%.⁹ Whereas, of the 260 patients on an LTMD (ie, montelukast, zafirlukast, or zileuton), only 17.7% of patients had a decrease in FEV_1 of greater than 20%.⁹ No benefit was seen with systemic steroids in this population; no comment was made on the change in rates of extrarespiratory symptoms, which were observed in less than 20% of the total study population. Further work has shown that on occasion LTMDs can completely prevent reaction symptoms during an oral aspirin challenge.¹⁰ These effects of LTMDs support our current understanding of the cysteinyl leukotriene (cysLT) derangements in AERD and are covered in greater detail later.

Changing the route of aspirin exposure from oral to intranasal decreases the severity of the respiratory and extrarespiratory symptoms observed during the aspirin challenge. Because the soluble form of aspirin, lysine aspirin, is not available in the United States, the standard intranasal protocol uses intranasal ketorolac over 4 doses before completing the protocol with oral aspirin at 60 mg, 100 mg, 160 mg, and 325 mg.¹¹ A study of 100 patients desensitized using this intranasal ketorolac protocol demonstrated that it can be a safer way of inducing desensitization when compared with 100 patients desensitized using only oral aspirin. Intranasal ketorolac decreased the mean decrease in FEV_1 (average decrease of 13.4% in oral challenge vs 8.5% in intranasal) and decreased the percentage of patients who developed extrapulmonary reactions (23% with intranasal ketorolac vs 45% with oral challenge), particularly

laryngospasm (7% vs 19%; $P = .02$) and gastrointestinal reactions (12% vs 33%; $P = .001$).⁶ Thus, intranasal protocols increase the safety of desensitizations while accomplishing the goal of a desensitized state.

Intranasal lysine aspirin can also be used for aspirin challenge and similarly provokes diminished rates of lower respiratory and extrarespiratory symptoms than seen with oral protocols. In a study of 131 patients who were first challenged with lysine aspirin to confirm the diagnosis, and if negative were then challenged with oral aspirin, a small subset (12%) reacted only to the oral aspirin challenge and not to the intranasal lysine aspirin challenge. Of those who reacted to the lysine-aspirin challenge, 100% developed nasal symptoms, though only 21 (18%) developed any lower respiratory symptoms. Only 2 (<2%) developed a decrease in FEV₁ of more than 20%, and only 7 patients (6%) developed any symptoms outside of the respiratory tract, which included facial itching, urticaria, and mild facial angioedema, whereas no gastrointestinal symptoms were reported.⁷ This finding suggests that decreasing the systemic absorption of the COX-1 inhibitor leads to decreased systemic symptoms without compromising the ability to bring about a desensitized state. Moreover, it highlights a role for both local nasal and systemic effector cells in patients with AERD.

PATHOPHYSIOLOGY OF ASPIRIN-INDUCED REACTIONS

The breadth of clinical symptoms noted during aspirin-induced reactions parallels the breadth of the clinical spectrum seen at baseline in patients with AERD. This clinical variation among patients, even those with a disease phenotype as distinct as AERD, suggests that the precise cellular and biochemical perturbations that cause the aspirin-induced reactions may vary from patient to patient. Like many complex medical conditions, a multitude of interconnected derangements have been reported in the cellular and mediator signaling cascades in AERD ([Table 1](#)). The identification of a single pathologic mechanism remains elusive and frankly frustrating for those researchers who study this disease. Fortunately for researchers, clinicians, and patients with AERD, progress has been made from all angles in recent years; we currently have a sufficient framework on which to eventually build the complete story. The lipid mediators, including cysLTs and prostaglandins, and the effector cells, including mast cells (MCs) and eosinophils, of the innate immune system are the central players that, with help from platelets, trigger the dramatic physiologic changes that occur during aspirin-induced reactions.

Table 1
Mechanisms of aspirin-induced reactions

Cells	Mediators
• Mast cell ◦ Acute activation during reactions • Eosinophil/basophil ◦ Acute influx into respiratory tissue during reactions • Platelets ◦ Increased platelet activation and platelet-leukocyte adhesion at baseline, which may contribute to leukotriene production and reaction symptoms	• CysLTs ◦ Increase acutely during reactions • Prostaglandin D₂ ◦ Increases acutely in the most severe patients during reactions • Prostaglandin E₂ ◦ Often decreases during reactions • Histamine and tryptase ◦ Increase acutely during reactions, especially in patients with systemic manifestations

Lipid Mediators

Leukotrienes

Early assessments of biological fluids during aspirin-induced reactions revealed the aberrant production of lipid mediators, specifically the cysLTs. Using leukotriene (LT) E_4 , the stable metabolite of the cysLTs, as a surrogate, patients with AERD demonstrate high baseline levels of cysLTs compared with healthy and aspirin-tolerant asthmatic (ATA) controls, which further increase in urine and nasal lavage during an aspirin-induced reaction. Baseline urine LTE_4 ($uLTE_4$) is almost 10-fold higher in AERD than in patients with ATA, and then increases 2- to 12-fold further following oral aspirin challenge in AERD.¹² CysLT levels predict symptom severity during aspirin-induced reactions,¹³ and higher baseline levels of cysLTs predict the involvement of both upper and lower respiratory symptoms during a reaction.¹⁴ The decrease in FEV_1 during oral aspirin challenge is associated with the degree of baseline $uLTE_4$ elevation and the extent to which $uLTE_4$ increases during reaction.¹³ This excess of cysLTs at baseline and during reaction has been recapitulated in multiple studies and forms our fundamental understanding of the pathobiology of this disease. Neither the exact mechanism that triggers the overproduction of cysLTs nor their cellular sources are known, but current data supports a role for impairments in prostaglandin (PG) E_2 production¹⁵ and the contributions of MC products^{16,17} and of platelet-leukocyte aggregates.¹⁸

Prostaglandins

In addition to the excessive production of cysLTs in AERD, it is recognized that PGD_2 is also overproduced. Multiple studies have demonstrated that patients with AERD have high levels of PGD_2 or one of the PGD_2 metabolites at baseline when compared with healthy and ATA controls.^{19,20} These values increase on exposure to aspirin, with significant increases from baseline noted at the onset of clinical reaction in both plasma¹⁹ and urine.^{20,21} Similar to $uLTE_4$ levels, baseline urinary tetranor- PGD -M levels, one of the dominant urinary PGD_2 metabolites, predict the decrease in FEV_1 during aspirin-triggered reactions and the increase in urinary tetranor- PGD -M during reactions correlates with symptom severity.⁸ Recent work established the connection between extremely high levels of PGD_2 during an aspirin-induced reaction and the development of extrarespiratory symptoms, specifically rash and gastrointestinal distress, including pain, nausea, and diarrhea, which can make it more difficult for patients to successfully complete their aspirin desensitization.⁸ These data suggest that PGD_2 contributes significantly to the symptoms seen at baseline and during aspirin-induced reactions in AERD, which is not surprising when we consider the known biological effects of PGD_2 . The PGD_2 metabolite 9a,11b- PGF_2 is bronchoconstrictive,²² which occurs through its signaling at the T prostanoid (TP) receptor. PGD_2 is also a chemoattractant for eosinophils, basophils, and innate lymphoid type 2 cells through the chemokine receptor homologous molecule expressed on Th2 lymphocytes (CRTH2).²³ The increase in PGD_2 production during aspirin-induced reactions inversely correlates with a decrease in peripheral blood eosinophils, likely indicating chemotaxis of CRTH2⁺ cells to the respiratory tissue.²⁰ Additionally, the positive correlation between nasal symptoms scores and PGD_2 production and the inverse correlation between FEV_1 and PGD_2 production seen during aspirin-induced reactions highlight the contribution of PGD_2 to reaction-induced symptoms.

Three major questions remain about PGD_2 generation in AERD:

1. What is the cellular source of PGD_2 ?
2. What causes the overproduction of PGD_2 at baseline in some patients with AERD?

3. How is PGD₂ being produced during aspirin-induced reactions, in the face of pharmacologic inhibition of COX-1?

The current working hypothesis is that MCs are the primary source of PGD₂ in AERD. This logical conclusion is built on the knowledge that PGD₂ is the primary COX product of MCs and that acute MC activation during aspirin-induced reactions is assumed due to the simultaneous detection of tryptase, histamine, and cysLTs, all canonical MC products.^{16,17} Further support for the role of the MC in AERD is discussed later. Eosinophils, which are abundant in the local respiratory tissue, are another potential source of PGD₂. Eosinophils do have the relevant terminal PGD₂ synthase, HPGDS, but express much less *HPGDS* transcript on a per-cell basis than do MCs.²⁰ It has been hypothesized that the ability of cells to generate excessive amounts of PGD₂ during aspirin-induced reactions when the COX-1 enzyme activity is pharmacologically inhibited is related to residual COX-2 activity. However, current evidence in subjects with ATA demonstrates that COX-2 inhibition does not suppress PGD₂ production or its downstream metabolites,²⁴ suggesting that PGD₂ production, at least in patients with ATA, is under COX-1 enzymatic regulation. Therefore, though the mechanism of PGD₂ overproduction remains elusive, it is hoped that the results of human studies using selective PGD₂ receptor antagonists will at least provide us with more conclusive evidence for the role of PGD₂ in AERD.

In addition to the elevation of PGD₂ during aspirin-induced reactions, the suppression of COX-1-derived PGE₂ following aspirin ingestion may be a key component underlying aspirin-induced reactions in AERD. Impairments in all steps along the PGE₂ signaling pathway, including COX-2, mPGES-1, and the EP₂ receptor, have been reported by multiple groups on multiple tissue and cell types from subjects with AERD.^{25–30} These impairments collectively are suspected to limit the ability of tissues/cells to upregulate PGE₂ production during times of inflammation, making patients with AERD particularly sensitive to COX-1 inhibition. Supporting these *in vitro* observations are 2 findings: (1) 15 minutes after bronchial aspirin challenge, PGE₂ levels in the bronchoalveolar lavage (BAL) decreased significantly in 11 patients with AERD³¹ and (2) pretreatment with inhaled PGE₂ prevented the aspirin-induced increase in uLTE₄ and the aspirin-induced decrease in FEV₁ during aspirin challenge.¹⁵ PGE₂ does serve as a negative regulator of 5-lipoxygenase (LO) through cyclic AMP/protein kinase A (PKA)³²; an aspirin-induced decrease in PGE₂ production, even locally, may be one driving factor for the excessive production of 5-LO products, specifically cysLTs. However, PGE₂ levels measured in the urine and nasal lavage during aspirin-induced reactions remain unchanged, suggesting that PGE₂ is not the sole mediator responsible for these reactions. Urinary PGE₂ levels do not decrease during aspirin-induced reactions^{8,33}, but there are significant amounts of PGE₂ generated in the kidney tubules, so urinary measurement may not be the best assessment of changes in respiratory PGE₂. In a small cohort of 5 patients with AERD, aspirin ingestion did not cause a decrease in nasal PGE₂ levels even when their nasal cysLT levels did go up,³⁴ so a simple shunting mechanism is not likely to be the complete answer.

Aspirin use, independent of disease state, has direct effects on other lipid mediators, notably thromboxane (TX) A₂ and prostacyclin.³⁵ TXA₂ is a potent bronchoconstrictor,³⁶ mediating its effects through the TP receptor. Additionally, TXA₂ supports PKA homeostasis, which in turn is critical for LTC₄ synthase levels and cysLT synthesis.³⁷ TXA₂ metabolites go down during reaction in most patients with AERD,³⁸ but those who demonstrate excessive PGD₂ production during a reaction similarly demonstrate TXA₂ production, as measured by an increase in the urinary metabolite 11-dehydroTXB₂.⁸ The TP receptor, which mediates bronchoconstriction and

through which both PGD₂ metabolites and TXA₂ signal, likely plays a key role in the lower respiratory symptoms that are so often observed during aspirin-induced respiratory reactions in patients with AERD. Early phase clinical trials of a selective TP receptor antagonist in AERD³⁹ are ongoing and should better inform our understanding of the importance of this signaling pathway.

Cells

During an aspirin-induced reaction in patients with AERD, we have the ability to directly and indirectly assess changes in resident and infiltrating effector cells to inform our understanding of this inflammatory state. The role of MCs, eosinophils, basophils, and platelets have been the primary focus of current research in AERD to date; current evidence supports a role for each cell in mediator generation or migration to the respiratory tissue during aspirin-induced reactions.

Mast cells

MCs are long-lived innate immune cells in the tissue that play a central role in AERD. In a study of 8 patients with AERD, aspirin ingestion caused an increase in nasal tryptase, histamine, and cysLTs; pretreatment with oral zileuton, an inhibitor of 5-LO, blocked both the nasal symptoms and the increase in nasal tryptase and cysLTs. This finding strongly suggests that MCs are activated during the nasal response to aspirin and that 5-LO products are important for the development of nasal symptoms and for the further release of MC mediators.¹⁷ Tryptase and histamine can also be released systemically, but this tends to be most pronounced in patients with extrapulmonary symptoms.⁴⁰ The use of sodium cromolyn to successfully inhibit aspirin-induced reactions further emphasizes the importance of MCs.⁴¹ However, there are no differences in the numbers of MCs within the nasal polyp tissue of patients with AERD²⁰; there is no evidence that MC activation occurs through an immunoglobulin E-dependent process in AERD. Therefore, we are left to question why MC activation occurs following aspirin ingestion in AERD and not ATA or healthy controls. One possible explanation returns us to the impairment in PGE₂ production observed in subjects with AERD. PGE₂ serves as an inhibitor of MC activation,⁴² and the loss of this brake following the inactivation of COX-1 may lead to unfettered MC activation.

Eosinophils/basophils

Chronic eosinophilic sinusitis is one hallmark of AERD, and eosinophils are likely drivers of the chronic inflammatory state in the respiratory tissue. Additionally, many patients with AERD demonstrate mild to moderate chronic peripheral blood eosinophilia.^{20,43} Less is known about basophils in the chronic state. During an aspirin-induced reaction, an influx of both eosinophils and basophils can be demonstrated into the nose⁴⁴ and into the BAL following an inhaled aspirin challenge.³¹ Correspondingly, a decrease of peripheral blood eosinophils within 1 hour of the onset of reaction symptoms following aspirin challenge has been observed.^{20,38} This migration of CRTH2⁺ cells from the blood stream to the respiratory tissue when PGD₂ levels are increasing suggests that the effector cells are responding to the PGD₂ gradient produced from within the local tissue.²⁰ Clearly defining the role of eosinophils and basophils as drivers of the acute reaction requires further investigation.

Platelets

Although the major function of platelet activation is the formation of thrombi in response to vessel wall injury, platelets also influence the function and activation of immune cells and can facilitate granulocyte recruitment into sites of tissue inflammation. There is an increasing body of evidence to suggest that platelet activation may be

specifically associated with the pathophysiology of AERD. Platelet activation is mediated by the binding of a diverse set of stimuli, and on activation the cell adhesion molecule P-selectin is expressed on the extracellular surface. This expression in turn facilitates platelet adhesion onto leukocytes, which constitutively express the ligand P-selectin glycoprotein ligand 1. This P-selectin-dependent adhesion to leukocytes allows for the priming and migration of these cells to inflamed tissues. Indeed studies have now shown that there is a higher percentage of platelet-adherent leukocytes in both the circulation and within the nasal polyp tissue of patients with AERD, compared with ATA controls, as well as an increased percentage of circulating platelets that express P-selectin in AERD.^{18,45}

Platelets also possess a range of inflammatory mediators that are released on activation and can act as a supply of newly synthesized lipid mediators. It may be that the platelets from patients with AERD are uniquely capable of inciting inflammation. For example, when compared with platelets from ATA controls, circulating platelets from patients with AERD generate an abnormal response when stimulated with aspirin *in vitro*, with a release of cytotoxic mediators and free radicals.⁴⁶ Platelets from patients with AERD also produce higher quantities of lipoxygenase products than do those from patients with ATA.⁴⁷ The increased presence of platelet-adherent neutrophils in AERD¹⁸ contributes to the overproduction of cysLTs seen in the disease, through the transcellular metabolism of arachidonic acid. Although neutrophils possess active 5-LO enzyme activity and can produce LTA₄ as a leukotriene intermediate, they lack the final LTC₄ synthase enzyme required for the production of cysLTs and cannot, on their own, convert arachidonic acid into cysLTs. Platelets lack 5-LO but do express LTC₄ synthase. However, when neutrophils and platelets are in close contact through P-selectin-dependent adhesion, excess LTA₄ released from the neutrophils can be converted into cysLTs by the platelet's LTC₄ synthase, creating a transcellular mechanism for the release of additional cysLTs.¹⁸

Additional investigations are underway to determine if the levels of platelet activation or platelet-leukocyte adhesion further increase during aspirin-induced reactions, which would provide a clear indication that platelets are directly involved in the acute reactions.

Innate Immune Mediators

Two mediators of innate type 2 immunity, interleukin 33 (IL-33) and thymic stromal lymphopoietin (TSLP), have also recently been implicated in the pathogenesis of AERD and the aspirin-induced reactions. IL-33 and TSLP are cytokines largely derived from structural cells and are known to provide an initial response to viral infections. These cytokines can induce MC activation and eosinophil accumulation into inflamed respiratory tissues,^{48,49} and nasal polyps from patients with AERD have been found to strongly express both IL-33 and TSLP.^{20,50} Additionally, in a mouse model of AERD, pharmacologic blockade of either IL-33 or its receptor completely prevented the increase in lung resistance and release of MC mediators induced by inhaled aspirin challenge.⁵⁰ These early studies suggest that activation and amplification of the innate type 2 immune system may play a role in the underlying mechanisms that lead to the chronic inflammation and/or the acute aspirin-induced reactions in AERD.

SUMMARY

There are extensive data to support that the clinical symptoms observed during acute aspirin-induced reactions in patients with AERD are largely due to both the sudden release of proinflammatory lipid mediators, particularly cysLTs and PGD₂, and the

rapid influx of effector cells into the respiratory tissues. To date, the data also strongly suggest that these lipid mediators are primarily produced by MCs, with likely contributions by platelet-leukocyte aggregates as well. However, there are many remaining questions regarding the underlying pathophysiologic mechanisms of these reactions. Neither the primary insult that causes the initial chronic disease process to begin nor the acute trigger of aspirin-induced reactions are known. Research is underway to further elucidate the mechanisms of reaction, in the hopes that understanding more about the pathogenesis of the COX-1 inhibitor-induced reactions will guide us to a better understanding of the disease overall and to the development of better treatments for our patients.

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