

EDITORIAL

The role of COX-1 and COX-2 in asthma pathogenesis and its significance in the use of selective inhibitors

Cyclooxygenases and arachidonic acid biotransformations

Arachidonic acid (AA), a 20-carbon fatty acid containing four double bonds, is liberated from the phospholipids by phospholipase A₂, following activation by diverse stimuli [1,2]. It is then converted by prostaglandin G/H synthases, which have both cyclooxygenase (COX) and hydroperoxidase (HOX) activity, to the unstable intermediate prostaglandin H₂. PGH₂, in turn, serves as the substrate for tissue-specific isomerases that transform it to prostaglandins and thromboxane A₂ (TXA₂). These autocoidal lipid mediators activate specific G-coupled, cell-membrane receptors. Some of the actions of individual prostanoids in the respiratory tract are indicated in Fig. 1. COX may also be involved in the synthesis of isoprostanes, which are isomers of prostaglandins formed by free radical oxygenation of AA. Isoprostanes are of interest as markers of oxidant injury and also as physiological analogs of prostaglandins.

It is now recognized that there are two related, but distinct, genes products of prostaglandin G/H synthase, colloquially termed COX-1 and COX-2. COX-1, expressed constitutively in most tissues, is thought to release prostaglandins involved in 'housekeeping' functions, such as the maintenance of gastrointestinal tract and vascular homeostasis. COX-2 is undetectable in most tissues. It can be up-regulated by bacterial lipopolysaccharides, cytokines, growth factors and tumour promoters, suggesting its relevance to inflammation and cancer. While the catalytic activities and tertiary structures of COX-1 and COX-2 are remarkably similar [2], COX-2 has a broader specificity for substrates because the hydrophobic channel leading to the active site of this enzyme is more accommodating. However, distinguishing COX-1 as a constitutive enzyme and COX-2 as an inducible enzyme, responsible for the formation of prostanoids in disease, is an oversimplification of the biologic reality [3].

Non-selective and selective COX inhibitors

Aspirin and other non-steroid anti-inflammatory drugs (NSAIDs) inhibit both COX-1 and COX-2. They differ in inhibitory potency, and their pharmacokinetic and pharmacodynamic properties determine their action in individual patient. Depression of prostaglandin synthesis is thought to account for both therapeutic and adverse effects associated with the administration of NSAIDs [4]. Inhibition of COX-2 has been more implicated in ameliorating inflammation, whereas the inhibition of COX-1 has been related to adverse effects in the gastrointestinal tract, such as dyspepsia, abdominal pain and, less frequently, gastric and duodenal perforation and bleeding. Based on these premises selective COX-2 inhibitors, termed coxibs, have been

developed. Two large clinical trials [5,6] performed with the first coxibs (rofecoxib and celecoxib) demonstrated that they are as effective as classical NSAIDs, but cause significantly fewer gastrointestinal adverse events.

It has been hypothesized that COX-2 selective drugs could unfavourably influence the haemostatic balance by inhibiting endothelial prostacyclin (PGI₂) production, while sparing platelet thromboxane A₂ synthase [3,7]. This matter has gained a lot of recent publicity following a debatable meta-analysis published by Topol et al. [8]. On the other hand, a more recent study [9] demonstrated that up-regulation of COX-2 and prostaglandin E (PGE₂) synthase by activated macrophages is associated with acute ischaemic syndromes, possibly through a metalloproteinase-induced plaque rupture. This study raised the possibility that the selective COX-2 inhibitors might provide a novel form of therapy for atherosclerotic plaque stabilization.

Cyclooxygenases and their products in the respiratory tract

Gained by the gene-targeting strategies, *PGHS-1* or *PGHS-2* knockout mice have recently been studied in a standard model of allergic airway disease [10]. Mice deficient in cyclooxygenase activity had normal lungs. In this animal model of ovalbumin-induced asthma, COX-1 seemed more important in lung protection, probably due to more efficient coupling with constitutive cytosolic isomerase producing PGE₂. When compared to the bronchoalveolar lavage (BAL) fluid of control animals, ovalbumin-sensitized animals had an increase in the number of eosinophils and lymphocytes. The magnitude of cellular influx was much greater in COX-1-deficient than COX-2-deficient animals. This was also reflected in lung sections studies. In COX-2-deficient animals, inflammatory cells accumulated more focally than in those who were COX-1-deficient, and occupied mostly perivascular and peribronchial areas. Structural findings corresponded to immunoblots and biochemical results. Expression of both COX-1 and COX-2 in control lung was constitutive, but PGE₂ in BAL fluid decreased only in COX-1 knockout mice. Increased respiratory resistance and hyper-responsiveness to metacholine were found in ovalbumin-sensitized, COX-1-negative animals [10].

Results of animal studies seem to agree with clinical findings. COX-1 and COX-2 immunoreactivities were reported in bronchial mucosa of normal and asthmatic lungs [11]. Both enzymes contribute to prostaglandins synthesis, and their expression increases in asthma and sputum cells as well as in the bronchial epithelium [12]. In human bronchial smooth muscle cells, COX-2 expression is easily induced by pro-inflammatory interleukin (IL)-1 β , leading to increased PGE₂ formation [13]. Two human prostaglandin E synthases (PGES)s have recently been

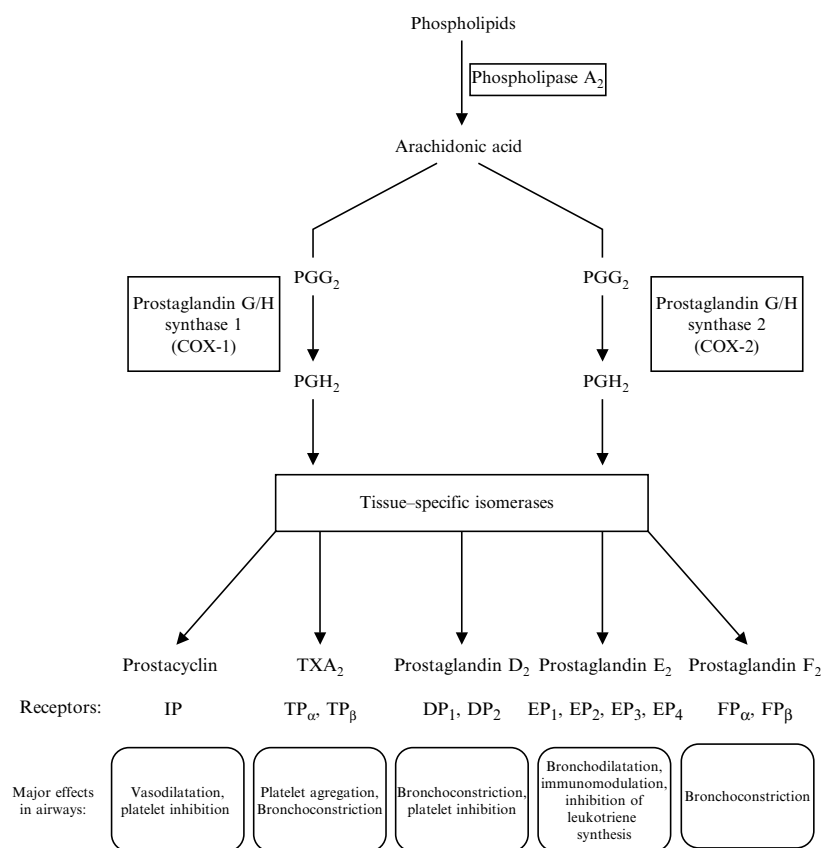


Fig. 1. Arachidonic acid biotransformations via COX pathways in relation to airway function.

identified; a microsomal, glutathione-dependent, inducible enzyme [14] and a cytosolic constitutive one [15]. In the lungs, the mRNA signal of the microsomal enzyme was moderate, and weaker than in the placenta or prostate. In macrophages, induction of COX-2 was parallel to the induction of microsomal PGES [16]. The pharmacological action of PGE₂ is mediated through four different receptors (EP₁–EP₄), with EP₃ having several splicing variants. It has been proposed that endogenous PGE₂ has an important anti-inflammatory and bronchoprotective role in human airway [17]. In clinical trials, inhalation of PGE₂ prevented allergen-induced bronchoconstriction and attenuated both immediate and late-phase of asthma and inflammation by the reduction of eosinophil counts in the sputum [17,18]. PGE₂ was also effective in preventing exercise-induced asthma [19], but it did not change bronchial hyper-reactivity to metacholine. Profound protective effects of PGE₂ were noticed in aspirin-induced asthma [20].

Prostaglandin D₂ synthase exists also as two isoenzymes. In the bone marrow-derived cells PGD₂ synthase belongs to glutathione S-transferases family [21]; it is expressed in the lung mast cells. The other PGD₂ synthase is expressed in the brain and shares homology with α₁-microglobulin and with complement C8; it belongs to the family of hydrophobic ligands carriers – lipocalins [22]. Mast cells are the main source of PGD₂ in the lung, releasing it upon activation by immunoglobulin (Ig) E-dependent and -independent mechanisms. It has been demonstrated that inhaled PGE₂ decreases allergen-precipitated release of PGD₂ into the BAL fluid of patients with asthma; it also decreases the release of cys-LTs, though not of tryptase [23].

Prostaglandin D₂ receptor (DP) is activated by PGD₂, and to a lesser extent by PGE₂ or PGF_{2α}. It stimulates adenylyl cyclase and increases intracellular calcium concentration through direct influx of extra-cellular calcium, inducing bronchial constriction, mucus secretion and vascular permeability. In the mouse model, knockout of the DP receptor greatly reduced bronchial hyper-reactivity induced by ovalbumin-sensitization. Reduced concentrations of T helper (Th)2-type cytokines (IL-4, IL-5, IL-13) in BAL fluid correlated with the decreased influx of eosinophils and lymphocytes in DP-negative animals, repeatedly challenged with ovalbumin [24]. Expression of haematopoietic PGD₂ synthase in human mast cells is regulated by an activator protein (AP)-2 transcription factor and augments upon stimulation with phorbol ester *in vitro* [25]. Perhaps reciprocal control of COXs-derived PGH₂ metabolism consists on haematopoietic PGD₂ synthase suppression by PGE₂. PGE₂ also positively modulates the expression of COX-2 in cell culture of human bronchial smooth muscles [26].

Allergic and pseudoallergic reactions to COX inhibitors

Classifying allergic and pseudoallergic reactions to aspirin and other anti-inflammatory and analgesic drugs have met with considerable difficulty. One of the reasons is our inability to establish *in vitro* tests to identify patients who experience the reactions. Furthermore, there is a spectrum of target organ responses to NSAIDs, such that the same drug may induce different types of reactions in different organ systems in the

same or different individual. A proposal for classification of these reactions has been recently published [27]. Here we discuss briefly two most common of them in relation to coxibs. These are: aspirin-induced asthma (AIA) and aspirin-induced urticaria/angioedema.

AIA; a distinct clinical syndrome [28], remains largely under-diagnosed. It affects about 8–10% of adult asthmatics, and this percentage rises in severe asthma. Thus, in a recent study [29], 16% of British patients with continuous asthma were found to suffer from aspirin intolerance. Over 25 years ago it was proposed [30] that AIA does not have an allergic background, but that the attacks are precipitated by the inhibition of COX by aspirin-like drugs. Confirmation of this proposition by various groups led to the formulation of the cyclooxygenase theory [31]. At the biochemical level AIA is characterized by chronic overproduction of cys-LTs, the levels of which rise further following aspirin ingestion. The key enzyme, LTC₄ synthase is over-expressed in the bronchial mucosa [32], which can be explained, in part, by the genetic variability of the LTC₄ synthase promoter [33].

The evidence is rapidly growing that coxibs are well tolerated by AIA patients [34–36]. More data exist for rofecoxib than celecoxib, though the latter seems to match the former in high tolerability. This high tolerance differentiates coxibs from nimesulide or meloxicam drugs known to inhibit COX-2 more than COX-1; they produce bronchoconstriction at higher doses. In a double-blind, controlled study of AIA patients [34], rofecoxib contrary to aspirin, precipitated neither clinical symptoms nor raised cys-LT production. These findings corroborate observations on diminished COX-2 expression and activity of nasal polyps from AIA patients [37–39], and they support the concept of PGE₂ brake as an important factor in response to NSAIDs [40]. The pharmacological action of COX-1 inhibitors would enhance depletion of this protective prostaglandin, down-regulated because of COX-2 deficiency. While this concept still awaits to be proved, possibly in a study of the patients' airway cell cultures, the available evidence indicates that one can safely prescribe coxibs to the patients with AIA.

In NSAID-induced urticaria/angioedema cross-reactivity with COX-1 inhibitory NSAID occurs in about a third of patients, when the hives are under control, and up to two thirds when the hives are active. This differentiates it from urticaria reactions to a single NSAID, some of which are clearly of an allergic background [27]. The mechanisms of the cutaneous reactions to analgesics may vary between patients, and are generally poorly understood. Lack of *in vitro* tests and scarcity of centres performing provocation tests often lead physicians to deny any treatment with NSAID to patients with urticaria/angioedema, especially if they give history of the drug sensitivity. The study of Maria Luisa Pacor and her colleagues [41] is therefore, welcome since it provides evidence of the high tolerance of rofecoxib in a relatively large population of patients with a history of skin reactions to NSAIDs. The authors clearly acknowledge the limitations of their research, the most important being a lack of definite proof for the presence of NSAIDs-induced reactions at the time of the study. The highly positive results are, however, encouraging. If confirmed, in a controlled study using rofecoxib vs. aspirin, they would open new possibilities for anti-inflammatory treatment in patients with urticaria/angioedema, induced or exacerbated by aspirin-like drugs.

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