Arachidonic acid-derived eicosanoids in rheumatoid arthritis: implications and future targets

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Rheumatoid arthritis (RA) is an autoimmune articular disease associated with chronic inflammation of the joints. RA is characterized by excessive synovial proliferation, progressive joint destruction and immobility. Cell types including neutrophils, macrophages, T and B cells infiltrate the inflamed joint area and release excessive amounts of proinflammatory mediators, resulting in the persistence of inflammation and resultant synovial tissue destruction. The most prominent mediators known to play a pivotal role in initiation and progression of articular inflammation during RA include proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1, reactive oxygen radicals, growth factors and lipid mediators generated via arachidonic acid (AA) metabolism. A diverse range of lipid mediators generated via AA biosynthetic metabolism, and often referred to as eicosanoids, mediate a wide variety of physiological and pathological functions. Eicosanoids play a central role in the progression and chronic inflammation associated with RA by mediating proinflammatory actions. However, recent studies have also shown that some eicosanoids generated during cell–cell interactions may act as stop signals for inflammation and help in the resolution of inflammation. Current therapies to treat the symptoms of RA are also directed towards inhibition of key enzymes and mediators within the AA biosynthetic pathway. This review outlines the broad understanding of AA-derived eicosanoid mediators in relation to RA. Furthermore, potential future therapeutic targets within the AA metabolic pathway for the treatment of RA will be discussed.

Arachidonic acid metabolism in rheumatoid arthritis

Arachidonic acid (AA) metabolites, including prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), hydroxyeicosatetraenoic acids (HETEs) and lipoxins (LXs), are essential mediators involved in the pathophysiology of rheumatoid arthritis (RA). These eicosanoids, so named for the 20 carbons contained in AA, exert a broad range of pro- and/or anti-inflammatory functions at the site of RA. The first step of AA metabolism begins with the release of free arachidonate from cell membrane phospholipids by phospholipase (PL)A_2. Subsequently, a diverse range of metabolites are generated downstream of PLA_2 by multiple biosynthetic pathways, including cyclooxygenase (COX), lipoxygenase (LOX) and further sequential-specific terminal enzymes that are expressed in a cell-type and tissue-specific manner (**Figure 1**).

Cyclooxygenase

COX is one of the major enzymes within the AA metabolic pathway and has been investigated extensively in the field of RA research. COX converts AA to PGH_2 which is the common substrate for physiologically active PGs and TXs, including PGE_2, PGD_2, $PGF_{2\alpha}$ PGI_2 (prostacyclin) and TXA_2. COX exists in at least two isoforms, constitutive COX-1 and inducible COX-2 [1,2]. Most recently, a splice variant of COX-1 mRNA, retaining intron 1 and referred to by several names, including COX-3, COX-1b or COX-1v, has been described [3,4]. However, the existence and role of these remain questionable. In the synovial tissue of RA, expression of COX-2, but not COX-1, is upregulated in infiltrating mononuclear inflammatory cells, synovial fibroblasts and vascular endothelial cells in synovial sublining layers [5,6]. In addition, induction of COX-2 is observed in response to stimulation by proinflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α in cultured synovial fibroblasts from patients with RA [7–9]. These observations regarding COX-2 induction in articular tissues seem to be closely associated with the clinical efficacy of COX-2-selective, as well as nonselective, inhibitors in the treatment of RA.

Prostaglandins

Among the PGs, PGE_2 is one of the most prominent proinflammatory PGs associated with rheumatoid synovitis, and high concentrations of PGE_2 are detectable in the synovial fluid of

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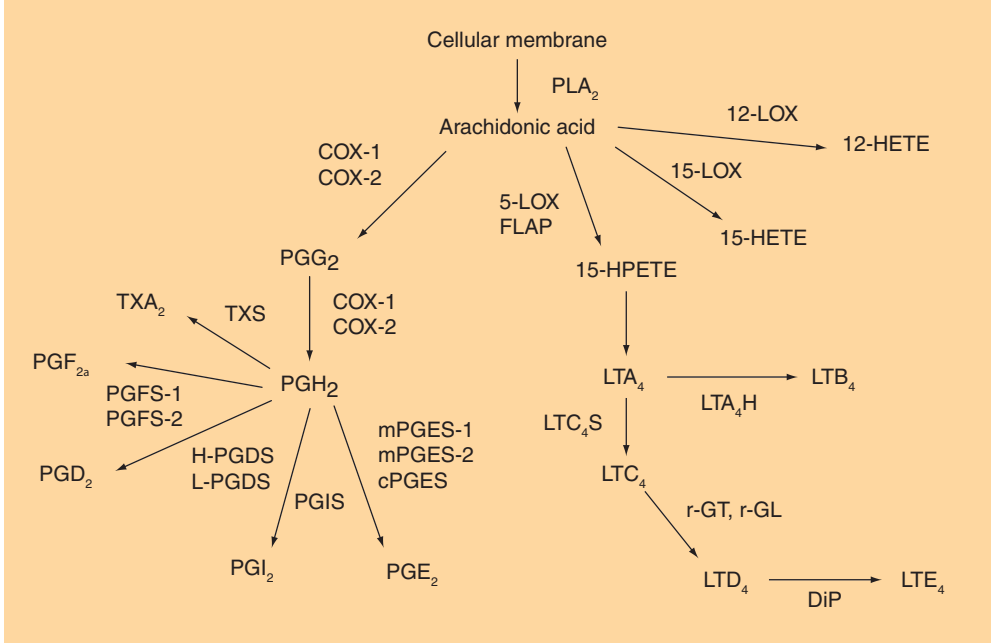
future medicine

Figure 1. Biosynthetic pathways in arachidonic acid metabolism.



COX: Cyclooxygenase; cPGES: Cytosolic PGE synthase; DiP: Dipeptidase; FLAP: 5-LOX activating protein; HETE: Hydroxyeicosatetraenoic acid; HPETE: Hydroperoxyeicosatetraenoic acid; H-PGDS: Hematopoietic PGDS; LOX: Lipoxygenase; LT: Leukotriene; LTA_4H: LTA_4 hydrolase; LTC_4S: LTC_4 synthase; L-PGDS: Lipocalin PGD synthase; mPGES: Microsomal PGES; PG: Prostaglandin; PGDS: PGD synthase; PGFS: PGF synthase; PGES: PGE synthase; PGIS: PGI synthase; PLA_2: Phospholipase A_2; r-GL: r-glutamyl leukotrienase; r-GT: r-glutamyl transpeptidase; TX: Thromboxane; TXS: Thromboxane synthase.

patients with RA [10]. At present, inhibition of PGE_2 production and action is most closely associated with a reduction in the pain and inflammation associated with RA. Ligand-activated cells, such as synovial fibroblasts and monocytes/macrophages, in synovial tissue are likely to be a primary source of PGE_2 in the joints of patients with RA [8,11,12]. Overproduction of PGE_2 in response to proinflammatory cytokine stimuli coincides with the upregulation of COX-2 expression at sites of inflammation [8].

PGE_2 exerts most of its actions through a family of G protein-coupled receptors (GPCRs) including EP1, 2, 3 and 4. It has been reported that RA synovial fibroblasts express EP2 and 4 receptors constitutively and that PGE_2 regulates the production of cytokines and growth factors, such as parathyroid hormone-related peptide, IL-6, macrophage colony-stimulating factor (M-CSF) and vascular endothelial growth factor (VEGF), through the activation of EP2 and 4 in IL-1-stimulated synovial fibroblasts [13]. PGE_2 is also a modulator of matrix metalloproteases (MMPs) and tissue inhibitors of MMPs (TIMPs) [14]. Furthermore, EP4 receptor-null mice, but not EP1, 2 and 3 receptor-null mice, have been shown to have reduced incidence and severity of collagen-induced arthritis (CIA) [15]. These reports suggest that modulating EP receptor signaling is a possible therapeutic strategy for treatment of RA.

PG terminal synthases

Terminal PG synthases are involved in the conversion of PGH_2 to active PGs and TXs downstream of COX. These enzymes are PGE synthase (PGES) for PGE_2, PGDS for PGD_2, PGFS for $PGF_{2\alpha}$, PGIS for PGI_2 and TXS for TXA_2, respectively. In the final step of PGE_2 biosynthesis downstream of COX, PGES specifically catalyzes the conversion of PGH_2 to PGE_2. The authors have previously reported that microsomal PGES (mPGES)-1, one of the PGES isozymes, is dramatically upregulated coordinately with COX-2 by stimulation with IL-1β and TNF-α in cultured synovial fibroblasts from RA patients [16–18]. Subsequently, it was demonstrated that mPGES-1 expression is correlated with disease activity in synovial lining cells, macrophages and fibroblasts in the

sublining, and mononuclear infiltrates and vascular endothelial cells in synovial tissues of patients with RA [19,20]. In addition, the potential importance of mPGES-1 in arthritis has been directly supported by the reduction of inflammatory and histopathological changes in CIA and collagen antibody-induced arthritis (CAIA) models in mPGES-1-null mice [21,22]. The absence of mPGES-1 appears equivalent to the absence of COX-2 in reducing PGE_2 production while preserving biosynthesis of alternate PGs. mPGES-1 might be an attractive future therapeutic target to control specifically the overproduction of PGE_2 associated with the inflammation of RA.

In contrast to inhibiting proinflammatory PGE_2, elevating the levels of anti-inflammatory PGs may also prove fruitful in the treatment of arthritis. PGDS is a key enzyme for the production of PGD_2 and its metabolite, 15-deoxy-Δ12, 14-PGJ_2 (15d-PGJ_2), that has been shown to exert anti-inflammatory/resolution effects on peripheral inflammation [23]. PGD_2 acts via at least two GPCRs, designated DP1 and DP2 receptors. It has been reported that retroviral expression of PGDS can suppress inflammatory responses in some animal models of inflammation [24,25]. In addition, 15d-PGJ_2 has an inhibitory effect on proinflammatory mediator production such as IL-1β, TNF-α, inducible nitric oxide synthase (iNOS), MMP-13 and MMP-1 via inhibition of inflammatory gene transcription by nuclear receptor activation in the articular component [26–28]. The mechanism by which 15d-PGJ_2 mediates its anti-inflammatory effects is not well known; however, recent studies suggest its ability to bind and activate peroxisome proliferator-activated receptor (PPAR)γ and activate anti-inflammatory mechanisms that help in the resolution of inflammation [29]. Furthermore, 15d-PGJ_2 also has a negative feedback effect on AA metabolic enzymes, such as COX-2 and mPGES-1, associated with the generation of proinflammatory PGE_2 [30–33]. In several studies, the anti-inflammatory effects of 15d-PGJ_2 seem to be partly associated with its pro-apoptotic effects on inflammatory cells. 15d-PGJ_2 inhibits the growth of RA synovial fibroblasts by apoptosis and also attenuates the inflammatory features, such as pannus formation and mononuclear cell infiltration, in a model of adjuvant-induced arthritis [34]. These studies suggest that 15d-PGJ_2 may be another useful therapeutic approach for inflammatory arthritis.

Lipoxygenase

Another alternative metabolism of AA occurs via the LOX enzyme, which results in the production of HETEs and LTs. LOX metabolic enzymes, including 15- and 12-LOX, yield 15- and 12-HETE, respectively, by metabolizing AA, whereas 5-LOX metabolizes AA to LTs [35]. 5-LOX metabolism of AA produces LTA_4 (an unstable metabolite), which is subsequently hydrolyzed by LTA_4 hydrolase (LTA_4H) to LTB_4, or LTC_4 by LTC_4 synthase in combination with reduced glutathione. LTC_4 is further converted to LTD_4 by γ-glutamyl leukotrienase (GL) and γ-glutamyl transpeptidase (GT), and converted subsequently to LTE_4 by dipeptidase [36,37].

Leukotrienes

LTs mediate most of their effects via LT receptors belonging to the GPCR superfamily. Cysteinyl LTs (CysLTs) including LTC_4, D_4 and E_4 bind to CysLT receptors ($CysLT_1$ and $CysLT_2$) [38], whereas LTB_4 acts by binding either to BLT_1 or BLT_2 receptors [39].

The role of CysLTs in RA is poorly understood. However, reports suggest that 5-LOX and its metabolic product, LTB_4, may play a key role in the pathophysiology of RA. Elevated levels of 5-LOX and LTB_4 have been observed in the serum, synovial fluid and synovium of the patients with RA [40,41]. One of the major proinflammatory actions of LTB_4 is its ability to act as a potent chemotactic factor for neutrophils [42]. Neutrophils are the predominant leukocytes present in synovial fluid. In addition, neutrophils in the inflamed joints are the major source of LTB_4 production in patients with RA [43]. Although neutrophils have long been considered as the major source of LTB_4, other cell types including macrophages, lymphocytes and mast cells also produce substantial amounts of LTB_4 under proinflammatory conditions [44–46]. A recent study demonstrated that the LTB_4 receptors BLT_1 or BLT_2 are expressed in the synovial tissues of patients with RA [47]. This study also showed that BLT_2 is the main receptor mediating the effects of LTB_4 in human RA synovial tissues.

Animal studies have also shown elevated levels of LTB_4 in the synovial fluid of carrageenan-induced arthritis and CIA, and it is a key mediator involved in the progression of CIA [42,48]. MK886, an inhibitor of LTB_4 synthesis, has been shown to decrease the articular inflammation, proinflammatory mediator production and joint destruction in a murine model of CIA [49].

Licofelone (ML3000), a dual inhibitor of the 5-LOX and COX pathways, has shown promising results in reducing joint destruction in an animal model of arthritis [50]; however, the efficacy of this and other similar drugs need further assessment in human RA.

Lipoxins

In 1971, Sir John Vane first demonstrated that the mechanism of action of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is due to the inhibition of COX and subsequent inhibition of PG production. However, in recent years, new endogenous mechanisms of aspirin action have been discovered. In addition to inhibiting PG production, aspirin has the ability to acetylate COX-2 and initiate the production of novel endogenous lipids called LXs or aspirin-triggered LXs (ATL) [51]. COX-2 in the presence of aspirin catalyzes the conversion of AA to 15-HETE, which is further metabolized to 15-epi LXs by the 5-LOX pathway. LXs, including LXA_4 and LXB_4, can also be generated endogenously during cell–cell interactions between products of 15- and 5-LOX or 5- and 12-LOX. LXs act via a specific GPCR known as LXA_4 receptor (ALXR) [52].

LXs mediate stop signals that help in the resolution of inflammation. In a number of animal models of inflammation, LXs have been shown to limit neutrophil chemotaxis, adherence and transmigration [53], inhibit eosinophil migration [54], inhibit superoxide anion generation [55] and attenuate the production of proinflammatory mediators such as TNF-α [56] and LTD_4 [57].

Knowledge of the role of these novel anti-inflammatory lipid mediators in RA is poorly understood to date; however, studies suggest they have therapeutic potential for counteracting inflammation. LXA_4 has been shown to decrease the levels of IL-1β-induced IL-6, IL-8 and MMP-3 in RA synovial fibroblasts [58]. In addition, LXA_4 has also been shown to stimulate TIMP-2 in human synovial fibroblasts [59]. Furthermore, treatment of neutrophils isolated from RA patients with ionophore A23187 results in elevated levels of LXs [60], further suggesting the anti-inflammatory/resolution effects of these lipid mediators.

Apart from LXs, aspirin treatment of human tissues *in vitro* and murine tissues *in vivo* have been shown to generate novel 17-*R*-hydroxy series docosanoids involved in limiting inflammation. These novel mediators are the oxygenated products derived from omega-3 polyunsaturated fatty acids. Since these mediators are generated during the resolution phase of inflammation, they are termed resolvins [61].

Efficacy of NSAIDs for the treatment of RA

NSAIDs act by inhibiting COX activity and are used extensively for the management of pain, swelling and stiffness in RA [62]. Nonselective or traditional NSAIDs, including indomethacin, ibuprofen and naproxen, although effective in the management of pain associated with RA, are associated with a number of gastrointestinal and renal side effects as these drugs inhibit both COX-1 and -2 activity and inhibit the production of PGs and TXs [63]. Since PGs generated by COX-1 are vital for the maintenance of gastrointestinal and renal function, selective COX-2 inhibitors (COXIBs), including celecoxib and rofecoxib, were developed and used extensively for the treatment of RA and other inflammatory and painful diseases. Despite improved symptoms, clinical trials have demonstrated that use of selective COXIBs may lead to an increased risk of cardiovascular events [64–66].

These studies have raised several questions regarding the balance of safety and efficacy for these drugs in the treatment of inflammatory diseases, including RA. New targets within the AA metabolism are being identified to overcome the possible adverse effects associated with selective COX-2 inhibition. Since studies using mPGES-1-deficient mice reveal resistance to pain, arthritis and fever, selective mPGES-1 inhibitors are the most promising. However, no specific inhibitor of this enzyme is currently available.

Future perspective

Eicosanoids have been implicated as key mediators involved in the inflammatory symptoms of RA, particularly pain, stiffness and swelling. NSAIDs and COXIBs are used extensively for the treatment of RA, even in this era of biologic therapies. However, when one looks at the history of NSAIDs and COXIBs, more drugs have failed than succeeded. One example of this is that, of the three COXIBs indicated for the treatment of RA released since 1999, only one remains on the market. The current issues include ongoing gastrointestinal risk that, while reduced by several strategies, including co-administration of proton pump inhibitors or misoprostol and the use of COX-2-specific inhibitors, have not eliminated serious gastrointestinal events associated with these medications. Co-administration of low-dose aspirin,

whose use is quite frequent in patients with RA, especially in light of the increased cardiovascular risk in this disease, increases gastrointestinal toxicity. All of these drugs have renal effects, including increased blood pressure, which may contribute to long-term issues with cardiovascular adverse events. The cardiovascular issues continue to be troublesome and it cannot be concluded from current data that any drug that inhibits COX-2, whether nonspecific or specific, has no adverse effects.

The fact that the COX isozymes sit relatively high up in the biosynthetic pathway and lead to inhibition of all PGs make inhibition of these enzymes rather crude tools if indeed the desire is to inhibit the production or action of only one PG or block its actions. The current task is to devise new therapeutic tools to control the chronic inflammation associated with RA. More specific targeting of the eicosanoid pathway may allow for benefit, while minimizing the risks. As research progresses to provide evidence of the specific eicosanoids involved in inflammation and anti-inflammation aimed specifically at altering this balance, more targeted therapies will become possible. At present, inhibition of PGE_2 production and action seems most likely to treat the pain and inflammation of RA. Promoting increased levels of anti-inflammatory eicosanoids may also prove fruitful in the treatment of RA. These strategies may be useful for symptom modification or possibly in disease modification. At present, the potential adverse consequences of alternate treatment strategies remain unclear.

Executive summary

Cyclooxygenase

- Cyclooxygenase (COX) is mainly comprised of two isozymes, constitutive COX-1 and inducible COX-2.
- Induction of COX-2 expression has been observed in inflamed synovial tissues of rheumatoid arthritis (RA) patients with the disease-related pattern.

Prostaglandins

- Elevated levels of proinflammatory prostaglandin (PG)E_2 are observed in the synovial fluid of patients with RA.
- Microsomal PGE synthase (mPGES)-1 is expressed in correlation with the extent of disease activity in synovial tissues of patients with RA.
- Deletion of the PGE_2 biosynthetic enzyme and its receptor action have been demonstrated to reduce inflammatory and histopathological changes in arthritis animal models.
- Anti-inflammatory PGs, such as PGD_2 and 15-deoxy-Δ12, 14-PGJ_2, may be a useful therapeutic approach for inflammatory arthritis.

Lipoxygenase

- Lipoxygenase (LOX) metabolic enzymes, including 15- and 12-LOX, yield 15-hydroxyeicosatetraenoic acid (HETE) and 12-HETE, respectively, by metabolizing arachidonic acid, whereas 5-LOX metabolizes arachidonic acid to leukotrienes (LTs).

Leukotrienes

- Elevated levels of LTB_4 have been observed in the serum, synovial fluid and synovium of patients with RA.
- Inhibitors of LTB_4 synthesis have been shown to decrease articular inflammation, proinflammatory mediator production and joint destruction in animal models of arthritis.

Lipoxins

- Aspirin has the ability to acetylate COX-2 and initiate the production of novel endogenous lipids called lipoxins.
- Lipoxins mediate stop signals that help in the resolution of inflammation.

Nonsteroidal anti-inflammatory drugs

- Recent clinical trials have demonstrated that use of selective COX-2 inhibitors can lead to an increased risk of cardiovascular events.
- mPGES-1 is an attractive therapeutic target for RA disease.

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