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

Mohit Kapoor, Fumiaki Kojima & Leslie J Crofford†

†Author for correspondence
University of Kentucky, Department of Internal Medicine, Rheumatology Division, Room J 509, Kentucky Clinic, Lexington, KY 40536-0284, USA
Tel.: +1 859 323 4939; Fax: +1 859 257 8258; lcrofford@uky.edu

Keywords: COX, eicosanoid, inflammation, leukotriene, lipoxin, lipoxygenase, NSAIDs, prostaglandin E synthase, rheumatoid arthritis

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)A2. Subsequently, a diverse range of metabolites are generated downstream of PLA2 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 PGH2 which is the common substrate for physiologically active PGs and TXs, including PGE2, PGD2, PGF2α, PGI2 (prostacyclin) and TXA2. 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 pro-inflammatory 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, PGE2 is one of the most prominent proinflammatory PGs associated with rheumatoid synovitis, and high concentrations of PGE2 are detectable in the synovial fluid of
patients with RA, and these reports suggest that modulating EP receptor signaling is a possible therapeutic strategy for the treatment of RA.

**PG terminal synthases**

Terminal PG synthases are involved in the conversion of PGH2 to active PGs and TXs downstream of COX. These enzymes are PG synthase (PGES) for PGE2, PGDS for PGD2, PGFS for PGF2α, PGIS for PGI2 and TXS for TXA2, respectively. In the final step of PGE2 biosynthesis downstream of COX, PGES specifically catalyzes the conversion of PGH2 to PGE2. 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. Subsequently, it was demonstrated that mPGES-1 expression is correlated with disease activity in synovial lining cells, macrophages and fibroblasts in the

**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; LTA4-H: LTA4 hydrolase; LTC4-S: LTC4 synthase; L-PGDS: Lipocalin PGD synthase; mPGES: Microsomal PGES; PG: Prostaglandin; PGDS: PGD synthase; PGFS: PGF synthase; PGES: PGE synthase; PGIS: PGI synthase; PLA2: Phospholipase A2; r-GL: r-glutamyl leukotrienase; r-GT: r-glutamyl transpeptidase; TX: Thromboxane; TXS: Thromboxane synthase.
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 PGE2 production while preserving biosynthesis of alternate PGs. mPGES-1 might be an attractive future therapeutic target to control specifically the overproduction of PGE2 associated with the inflammation of RA.

In contrast to inhibiting proinflammatory PGE2, 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 PGD2 and its metabolite, 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2), that has been shown to exert anti-inflammatory/resolution effects on peripheral inflammation [23]. PGD2 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-PGJ2 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-PGJ2 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-PGJ2 also has a negative feedback effect on AA metabolic enzymes, such as COX-2 and mPGES-1, associated with the generation of proinflammatory PGE2 [30–33]. In several studies, the anti-inflammatory effects of 15d-PGJ2 seem to be partly associated with its pro-apoptotic effects on inflammatory cells. 15d-PGJ2 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-PGJ2 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 LTA4 (an unstable metabolite), which is subsequently hydrolyzed by LTA4 hydrolase (LTA4H) to LTB4, or LTC4 by LTC4 synthase in combination with reduced glutathione. LTC4 is further converted to LTD4 by γ-glutamyl leukotrienease (GL) and γ-glutamyl transpeptidase (GT), and converted subsequently to LTE4 by dipeptidase [36,37].

**Leukotrienes**

LTs mediate most of their effects via LT receptors belonging to the GPCR superfamily. Cysteinyl LTs (CysLTs) including LTC4, D4 and E4 bind to CysLT receptors (CysLT1 and CysLT2) [38], whereas LTB4 acts by binding either to BLT1 or BLT2 receptors [39]. The role of CysLTs in RA is poorly understood. However, reports suggest that 5-LOX and its metabolic product, LTB4, may play a key role in the pathophysiology of RA. Elevated levels of 5-LOX and LTB4 have been observed in the serum, synovial fluid and synovium of the patients with RA [40,41]. One of the major pro-inflammatory actions of LTB4 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 LTB4 production in patients with RA [43]. Although neutrophils have long been considered as the major source of LTB4, other cell types including macrophages, lymphocytes and mast cells also produce substantial amounts of LTB4 under proinflammatory conditions [44–46]. A recent study demonstrated that the LTB4 receptors BLT1 or BLT2 are expressed in the synovial tissues of patients with RA [47]. This study also showed that BLT2 is the main receptor mediating the effects of LTB4 in human RA synovial tissues.

Animal studies have also shown elevated levels of LTB4 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 LTB4 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 LXA₄ and LXB₄, 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 LX₄ 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], and attenuate the production of proinflammatory mediators such as TNF-α [56] and LTD₄ [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. LXA₄ 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, LXA₄ 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

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

<table>
<thead>
<tr>
<th>Prostaglandins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated levels of proinflammatory prostaglandin (PGE)(_2) are observed in the synovial fluid of patients with RA.</td>
</tr>
<tr>
<td>Microsomal PGE synthase (mPGES)-1 is expressed in correlation with the extent of disease activity in synovial tissues of patients with RA.</td>
</tr>
<tr>
<td>Deletion of the PGE(_2) biosynthetic enzyme and its receptor action have been demonstrated to reduce inflammatory and histopathological changes in arthritis animal models.</td>
</tr>
<tr>
<td>Anti-inflammatory PGs, such as PGD(<em>2) and 15-deoxy-(\Delta</em>{12}, 14)-PGJ(_2), may be a useful therapeutic approach for inflammatory arthritis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipoxygenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>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).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leukotrienes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated levels of LTB(_4) have been observed in the serum, synovial fluid and synovium of patients with RA.</td>
</tr>
<tr>
<td>Inhibitors of LTB(_4) synthesis have been shown to decrease articular inflammation, proinflammatory mediator production and joint destruction in animal models of arthritis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin has the ability to acetylate COX-2 and initiate the production of novel endogenous lipids called lipoxins.</td>
</tr>
<tr>
<td>Lipoxins mediate stop signals that help in the resolution of inflammation.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nonsteroidal anti-inflammatory drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent clinical trials have demonstrated that use of selective COX-2 inhibitors can lead to an increased risk of cardiovascular events.</td>
</tr>
<tr>
<td>mPGES-1 is an attractive therapeutic target for RA disease.</td>
</tr>
</tbody>
</table>
Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

14. First interesting work on prostaglandin (PG) E2 regulation.
20. First report on induction of microsomal PGE synthase (mPGES)-1 in rheumatoid arthritis synovial fibroblasts.
22. First study to show the positive feedback mechanism of mPGES-1 induction by PGE2.
26. First report to define the role of mPGES-1 in pain and inflammatory responses using mPGES-1 knockout mice.
Arachidonic acid metabolism in rheumatoid arthritis – REVIEW


• Interesting study focusing on apoptotic effects of 15-deoxy-Δ12,14-PGJ(2) in arthritis.


• One of the first reports on the role of lipoxins in arthritis.


• Interesting overview of the anti-inflammatory properties of resolvins.


**Affiliations**

- **Mohit Kapoor**
  University of Kentucky, Department of Internal Medicine, Rheumatology Division, Room J-509, Kentucky Clinic, 740 S. Limestone St., Lexington, KY 40536-0284, USA
  Tel.: +1 859 323 4939; Fax: +1 859 257 8258; mkapo2@email.uky.edu

- **Fumiaki Kojima**
  University of Kentucky, Department of Internal Medicine, Rheumatology Division, Room J-509, Kentucky Clinic, 740 S. Limestone St., Lexington, KY 40536-0284, USA
  Tel.: +1 859 323 4939; Fax: +1 859 257 8258; fkoji2@email.uky.edu

- **Leslie J Crofford**
  University of Kentucky, Department of Internal Medicine, Rheumatology Division, Room J-509, Kentucky Clinic, 740 S. Limestone St., Lexington, KY 40536-0284, USA
  Tel.: +1 859 323 4939; Fax: +1 859 257 8258; lcrofford@uky.edu

**One of the earliest clinical trials on the cardiovascular effects of celecoxib.**