

# The pathogenetic roles of arachidonate 5-lipoxygenase, xanthine oxidase and hyaluronidase in inflammatory diseases: A review

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## Abstract

Inflammation is a complex biological process essential for protecting the body from harmful stimuli. However, dysregulated or chronic inflammation can contribute to the pathogenesis of various diseases, including asthma, rheumatoid arthritis, and gout. Pro-inflammatory enzymes, such as arachidonate 5-lipoxygenase (A5-LOX), xanthine oxidase, and hyaluronidase, play key roles in the initiation, progression, and resolution of inflammation. A5-LOX catalyzes the production of leukotrienes, potent mediators of immune cell recruitment and activation, which are implicated in allergic and inflammatory responses. Xanthine oxidase contributes to oxidative stress and inflammation through the generation of reactive oxygen species during purine metabolism, and its inhibition offers therapeutic potential for diseases like gout. Hyaluronidase, by degrading hyaluronic acid, disrupts the extracellular matrix, leading to increased tissue permeability and exacerbating inflammation, as seen in conditions such as rheumatoid arthritis. This review examines the roles of these enzymes in inflammatory diseases and explores their potential as therapeutic targets. Inhibition of A5-LOX, xanthine oxidase and hyaluronidase present promising strategies for managing chronic inflammatory diseases, with ongoing research focused on developing more selective and effective enzyme inhibitors. Understanding the molecular mechanisms behind these enzymes may lead to novel, more precise treatments for inflammatory diseases, improving patient outcomes.

**Keywords:** Arachidonate 5-lipoxygenase, Enzyme inhibitors, Hyaluronidase, Inflammation, Inflammatory mediators, Pro-inflammatory enzymes, Therapeutic targets, Xanthine oxidase

## Introduction

Inflammation is a complex biological response of the immune system designed to eliminate harmful stimuli, such as pathogens, damaged cells, allergic or chemical irritants and toxic compounds, as well as to repair tissue damage. It serves as a protective mechanism that involves immune cells, blood vessels and various molecular mediators to neutralize the injurious stimuli and initiate the healing process. Erythema, edema, heat, pain, and altered function are the cardinal signs of inflammation [1-5]. In the absence of inflammation, wounds would fail to heal, infections would persist, and progressive tissue damage would ultimately threaten the survival of the host [2,6]. However, when inflammation becomes uncontrolled, it can lead to excessive tissue damage. If inflammatory proteins continue to accumulate throughout the body, they directly and indirectly cause various diseases. Inflammation is involved in the disease processes of allergies, arteriosclerosis, autoimmune diseases, dementia, eye diseases, and even certain types of cancer [4,5,7].

Pro-inflammatory enzymes play a pivotal role in initiation, progression and resolution of inflammation. These enzymes, including arachidonate 5-lipoxygenase (A5-LOX), xanthine oxidase, and hyaluronidase, are involved in the synthesis of key inflammatory mediators such as prostaglandins, leukotrienes, and reactive oxygen species (ROS), which regulate immune responses. Dysregulation or overactivation of these enzymes can lead to chronic inflammation, which is implicated in a wide range of inflammatory diseases [3,4,8,9]. This review explores the roles of A5-LOX, xanthine oxidase, and hyaluronidase in the pathogenesis of inflammatory diseases. Understanding the multifaceted contributions of these enzymes could provide novel insights into the development of more effective therapeutic strategies for managing inflammatory diseases.

## Acute and Chronic Inflammation

Acute inflammation is the immediate biochemical and cellular response of the body to harmful stimuli. At the onset of an infection, burn or other injury, immune cells are activated and inflammatory mediators, which are responsible for the clinical signs of inflammation, are released. Acute inflammation is initiated by resident immune cells already present in the injured tissues and continued with the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, immune system and various cells within the injured tissue [1,2,6]. During acute inflammatory responses, cellular and molecular events and interactions efficiently minimize the impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of acute inflammation. However, if uncontrolled, acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases [3].

Inflammation becomes chronic due to the persistence of the initiating factors and to a failure of mechanisms required for resolving the inflammatory response. Thus, chronic inflammation promotes whether directly or indirectly an increase in cell proliferation, an enhancement of inflammatory cell recruitment and excessive production of ROS, reactive nitrogen species (RNS) and active proteolytic enzymes [10]. Chronic inflammation leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [2,5]. It can lead to a multitude of diseases, including hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, osteoarthritis, gout, Alzheimer's disease and obesity, and can even cause cancers such as gallbladder carcinoma [11].

As a result of the local immune, vascular, and inflammatory cell responses to the infection or injury, clinical signs of inflammation including redness, increasing temperature, swelling, pain, and loss of function are emerged. However, not all five signs occur in every inflammation as some inflammations do not cause any symptoms. These clinical signs reflect the increased blood flow and capillary permeability, release of inflammatory mediators and leukocyte migration to the site of inflammation. Many different immune cells can take part in inflammation and they release different inflammatory mediators causing the narrow blood vessels in the tissue to expand and increase the blood flow to the inflamed area. Consequently, the inflamed area turns red and becomes hot. The

inflammatory mediators increase the permeability of the narrow vessels, allowing more defense cells to enter the site of inflammation. The defense cells also carry more fluid into the inflamed tissue and swelling occurs due to the accumulation of fluid. Inflammatory mediators stimulate nerve endings and increase the sensitivity to pain. When an inflamed limb can no longer be moved properly or when the sense of smell is worse during a cold or when it is more difficult to breathe while having bronchitis, it is known as loss of function and it occurs due to a combination of factors [1,3,12,13].

## Arachidonate 5-lipoxygenase Enzyme

Lipoxygenases are a family of non-heme, non-sulphur, iron cofactor containing dioxygenases (oxidoreductase enzymes) that are widely distributed in animals and plants. In human tissues, lipoxygenases are expressed in platelets, eosinophils, synovial fluids, neutrophils, colonic tissues, lung tissues, monocytes, and bone marrow cells [14]. Lipoxygenases catalyze the addition of molecular oxygen to polyunsaturated fatty acids, containing a cis, cis-1,4-pentadiene, such as linoleic, linolenic and arachidonic acid to yield unsaturated fatty acid hydroperoxides which can act as cell signaling agents [14-16]. In mammalian cells, lipoxygenases are the key enzymes which are involved in the biosynthesis of a variety of bioregulatory compounds such as hydroxyicosatetraenoic acids, leukotrienes, lipoxins, and hepoxylines [15,17]. Lipoxygenases are capable of generating lipid mediators such as leukotrienes and prostaglandins, which are capable of inducing inflammation and allergic symptoms. Lipoxygenase products play a key role in a variety of diseases such as bronchial asthma, allergic rhinitis, rheumatoid arthritis, cardiovascular diseases, tumor angiogenesis, and certain types of cancer [5,14,15,17,18]. Arachidonate 5-lipoxygenase (EC 1.13.11.34) is a member of the lipoxygenase family of enzymes. It involves in the biosynthesis of potent mediators of inflammatory disorders and allergic reactions. It is the key enzyme involved in the biosynthesis of leukotrienes and catalyzes the initial steps in the conversion of arachidonic acid to biologically active leukotrienes by introducing an oxygen molecule at the fifth carbon of the arachidonic acid chain [4,18,19].

## Leukotrienes

Leukotrienes are lipid mediators that play a central role in the recruitment and activation of immune cells such as neutrophils, eosinophils and macrophages, at the sites of inflammation. They are a group of signaling molecules produced in white blood cells (leukocytes) by the oxidation of arachidonic acid which is a polyunsaturated, 20-carbon fatty acid [19,20]. However, the over activity of A5-LOX or excessive production of leukotrienes is linked to the pathogenesis of a number of inflammatory diseases including asthma, allergic rhinitis, chronic bronchitis, rheumatoid arthritis, and inflammatory bowel disease as well as allergic reactions. Leukotrienes contribute to vasodilation, increased vascular permeability, and the promotion of pro-inflammatory cytokines and chemokines. They contribute to the signs and symptoms seen in inflammatory responses [14,19,20]. The esterified form of arachidonic acid is bound to the phospholipids of the cell membranes. Both immunological and non-immunological stimuli can activate the enzyme phospholipase A<sub>2</sub> and release arachidonic acid from the membrane phospholipids. The released arachidonic acid is oxidized by several distinct enzyme pathways including the cyclooxygenase pathway, which produces prostaglandins and thromboxanes, and

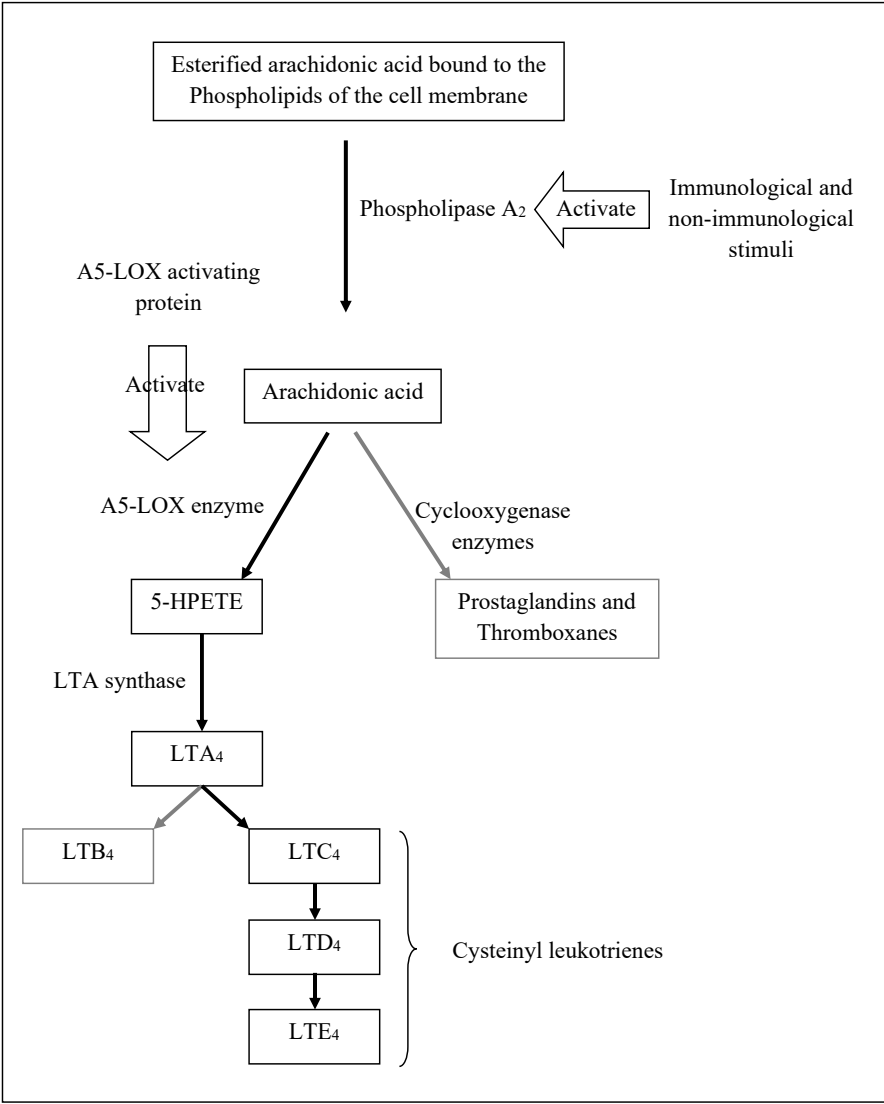
the lipoxygenase pathway, which produces leukotrienes and other intermediate compounds. In the lipoxygenase pathway, a nuclear membrane protein, A5-LOX activating protein, activates A5-LOX enzyme to oxidize arachidonic acid to 5 hydroperoxyeicosatetraenoic acid (5-HPETE) which is the precursor of the leukotrienes. Then 5-HPETE is converted into a series of leukotrienes and the nature of the final product varies according to the tissue. The enzyme LTA synthase catalyze the conversion of 5-HPETE to unstable leukotriene A<sub>4</sub> (LTA<sub>4</sub>) which is then converted to either leukotriene B<sub>4</sub> (LTB<sub>4</sub>) or leukotriene C<sub>4</sub> (LTC<sub>4</sub>). LTC<sub>4</sub> is actively transported out of cells and metabolized rapidly to leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and then to leukotriene E<sub>4</sub> (LTE<sub>4</sub>). LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> are referred to as the cysteinyl (Cys) leukotrienes because of their chemical structure. LTE<sub>4</sub> is either excreted in the urine or metabolized to a variety of biologically less active or inactive metabolites, including leukotriene F<sub>4</sub> (LTF<sub>4</sub>) [14,20]. The main pathways of formation of leukotrienes are shown in **Figure 1**.

**Arachidonate 5-lipoxygenase Inhibitors**

Since leukotrienes are considered as potent mediators of inflammatory and allergic reactions, inhibition of the lipoxygenase pathway is important in the prevention and treatment of a variety of inflammatory diseases. Therefore, A5-LOX is a potential target for the rational drug design to discover mechanism-based inhibitors for the treatment of inflammatory diseases [4,17,20]. Using A5-LOX inhibitors such as Zileuton and Montelukast in the treatment of asthma and allergies confirms the therapeutic significance of blocking the biosynthesis of the leukotrienes by the inhibition of A5-LOX, in the treatment of inflammatory diseases [20,21].

**Xanthine Oxidase Enzyme**

Xanthine oxidase (EC 1.17.3.2) is a member of the oxidoreductases class of enzymes. It is a molybdoflavin enzyme responsible for catalyzing the terminal two reactions in purine



**Figure 1.** Main pathways involved in the formation of leukotrienes.

degradation in primates; oxidation of hypoxanthine to xanthine and subsequently oxidation of xanthine to uric acid [5,22,23]. Uric acid undergoes no further metabolism in humans due to the absence of urate oxidase and is excreted by the kidneys and gastrointestinal tract [24,25]. Excessive production and/or inadequate excretion of uric acid results in hyperuricemia leading to the deposition of monosodium urate crystals in joints or kidneys causing gouty arthritis or uric acid nephrolithiasis [26,27]. Therefore, urate-lowering treatments either increasing the excretion of uric acid or reducing the uric acid production are required for the prevention and treatment of conditions associated with hyperuricemia. Urate-lowering agents include xanthine oxidase inhibitors, uricosuric agents and uricase agents [24,25].

During the oxidation of hypoxanthine and xanthine, and then xanthine to uric acid, superoxide anions and hydrogen peroxide, which are considered as ROS, are formed. Spontaneously or under the influence of superoxide dismutase enzyme, superoxide anions are converted into hydrogen peroxide and oxygen [24]. Due to the formation of ROS during the catalytic action (**Figure 2**), xanthine oxidase contributes to oxidative stress and plays a pathogenetic role in various forms of inflammatory diseases other than gout, several types of tissue and vascular injuries, and chronic heart failure [4,21,22].

### Xanthine Oxidase Inhibitors

Since xanthine oxidase plays an important role in the pathogenesis of inflammation via different pathways, inhibiting xanthine oxidase is considered as a target for the management of diseases associated with oxidative stress and inflammation [18]. Xanthine oxidase inhibitors are recognized as appropriate candidates for various therapeutic indications accompanied by the function of xanthine oxidase [5,21]. Inhibition of xanthine oxidase blocks the biosynthesis of uric acid and consequently reduces circulating levels of uric acid and reduces vascular oxidative stress. Xanthine oxidase inhibitors have been used as treatments for gout and uricemia. Inhibiting xanthine oxidase also has therapeutic potential for other inflammatory diseases where oxidative stress is a contributing factor. Allopurinol, oxypurinol, febuxostat, and probenecid are among the many known xanthine oxidase inhibitors [5,25]. However, they have side effects such as induction of drug resistance, inhibition of anticancer drug metabolism, and aplastic anemia [5]. Although allopurinol is the most commonly prescribed synthetic xanthine oxidase inhibitor widely used in the management of conditions associated with hyperuricemia and related inflammatory

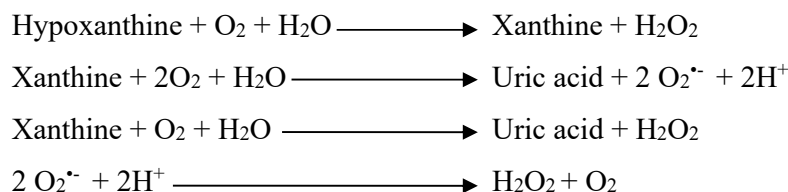
diseases, it is associated with several side effects including allergies, gastrointestinal distress, liver function abnormalities, and a fatal complication known as “allopurinol hypersensitivity syndrome” [22]. Therefore, there is an increasing demand for alternative xanthine oxidase inhibitors.

### Hyaluronic acid

Hyaluronic acid is a mucopolysaccharide composed of D-glucuronic acid and N-acetyl-D-glucosamine linked via the hexosaminidic bonds in  $\beta$  (1 $\rightarrow$ 4) linkages. It usually consists of 2,000 to 2,500 disaccharides to give a molecular mass between 106 and 107 Da and extended lengths of 2 to 25  $\mu$ m. It is generally found in the extracellular matrix, especially in soft connective tissues, skin, cartilage and in the capsule of some bacteria [5,28]. Hyaluronic acid plays an important role in several biological processes such as cellular adhesion, water balance and osmotic pressure regulation, mobility differentiation processes, where it acts as a lubricant and a shock absorber, and maintenance of tissue architecture [28,29]. It can regulate inflammatory responses by inhibiting the phagocytosis of macrophages [5].

### Hyaluronidase Enzyme

Hyaluronidase (EC 3.2.1.35) is one of the lysosomal enzymes which hydrolyzes mucopolysaccharides including hyaluronic acid [5,30]. Hyaluronidase cleaves the  $\beta$ -1,4-glycosidic bonds in hyaluronic acid, breaking it down into smaller oligosaccharides. This alters the structure and function of the extracellular matrix. Hyaluronidase involves in a number of physiological regulatory processes and pathological conditions including increased vascular permeability, destruction of extracellular matrix, embryogenesis, angiogenesis, inflammation, disease progression, wound healing, bacterial pathogenesis, diffusion of systemic venoms, and invasion of tumors. Hyaluronidase usually exists in an inactive form and is activated by antigens, compound 48/80 and certain metal ions including calcium ions [31]. The *in vivo* activation of hyaluronidase by metal ions leads to degranulation of mast cells, causing the release of inflammatory mediators. In inflammatory conditions, excessive hyaluronidase activity can promote tissue breakdown, increase vascular permeability, and facilitate the migration of inflammatory cells into tissues. Hyaluronidase also enhances the spread of infection by breaking down tissue barriers and facilitating the movement of pathogens. Hyaluronic acid is present in synovial fluid in joints as a viscous lubricating agent and hyaluronidase degrades it by lowering its viscosity and increasing the permeability. The



**Figure 2.** Formation of reactive oxygen species due to the catalytic actions of xanthine oxidase.

degradation of hyaluronic acid by hyaluronidase results in changes to tissue hydration, viscoelasticity, and cell signaling. Hyaluronidase is involved in conditions like rheumatoid arthritis, where its activity contributes to the breakdown of cartilage and the spread of inflammation. In rheumatoid arthritis, excessive degradation of hyaluronic acid by hyaluronidase causes depletion of its amount and molecular weight consequently producing arthritic symptoms [18,21,30].

## Hyaluronidase Inhibitors

Inhibition of hyaluronic acid degradation is critical and imperative in controlling hyaluronidase mediated pathological conditions. Hyaluronidase inhibitors serve as anti-inflammatory agents as well as anti-aging, antimicrobial, anticancer, antivenom/toxin, and contraceptive agents. Hyaluronidase inhibitors have therapeutic potential in limiting tissue destruction and inflammatory responses. The search for hyaluronidase inhibitors is important to identify new potent antiallergic and anti-inflammatory compounds which can serve as effective therapeutic agents [4,5,21,32].

## Conclusion

A5-LOX, xanthine oxidase and hyaluronidase are critical enzymes in the pathogenesis of inflammatory diseases, playing essential roles in the initiation, progression, and resolution of inflammation. These enzymes mediate inflammatory responses through the production of inflammatory mediators, such as leukotrienes and reactive oxygen species, and the degradation of extracellular matrix components. Dysregulation of these enzymes leads to the persistence and escalation of chronic inflammation, contributing to diseases such as asthma, rheumatoid arthritis, and gout. The therapeutic potential of targeting A5-LOX, xanthine oxidase, and hyaluronidase has already been demonstrated with inhibitors like zileuton, allopurinol, and various hyaluronidase inhibitors. These therapies have shown efficacy in reducing inflammatory symptoms and improving patient outcomes in certain conditions. However, there remains a void to be filled with more selective inhibitors which minimize side effects and enhance therapeutic benefits. Future research is crucial for further elucidating the complex mechanisms underlying these enzymes' roles in inflammation and for discovering novel compounds that can better control their activity. By advancing our understanding of these enzymes, we may be able to provide more effective, targeted treatments that not only manage inflammation but also offer long-term solutions for patients suffering from chronic inflammatory diseases.

## Conflict of Interest

The authors have no conflict of interest to declare.

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