

The role of aspirin-triggered lipoxins in the mechanism of action of aspirin

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Abstract

Few drugs have treated so many diseases, provided us with so much understanding of their pathogenesis, and tested our scientific creativity over the last 100 years as much as aspirin. Originally, the beneficial effects of aspirin were shown to stem from its inhibition of cyclooxygenase (COX 2)-derived prostanoids, fatty acid metabolites that modulate host defense and regulate the cardiovascular system. However, the inhibition of COX 2 enzyme activity and prostaglandin synthesis has never fully explained aspirin's repertoire of anti-inflammatory effects, leaving many questions pertaining to its true mechanism of action unanswered. Here, data from a series of comparatively recent experiments exploring aspirin's unique ability to acetylate the active site of inducible COX 2 and generate a family of lipid mediators called the epi-Lipoxins will be discussed in light of their ability to exert profound modulatory effects on the innate and adaptive immune systems.

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1. Introduction

In his informative account of the history of aspirin, Sir John Vane traces the use of aspirin back thousands of years to when salicylate, from which aspirin was derived, was a common constituent of many medicinal plants widely used to relieve pain and rheumatic fever [1]. Ancient Egyptian manuscripts contain references to the use of Myrtle leaves for rheumatic pain while Hippocrates applied extracts of poplar trees to treat eye infections and extracts of willow bark to alleviate fever and the pain associated with childbirth. Celsius also used extracts of willow bark to treat the four cardinal signs of inflammation—heat, redness, swelling and pain; Celsius' enduring description of the inflammatory response that survives to this day. It is no coincidence that Willow bark, Myrtle and Poplar trees all contain salicylate, a fact that did not escape the amateur clinical scientists like the Reverend Edmund Stone who, in 1763, administered a crude extract of willow bark to 50 of his

parishioners afflicted with rheumatic fever with notable success. This clinical milestone was repeated 100 years later in Dundee, Scotland, by T.J. MacLagan who showed efficacy in reducing inflammatory fever, pain and swelling. With the groundwork laid by earlier chemists in their attempts to elucidate the structure of medicinal willow bark, meadow sweet and wintergreen extracts, did Hermann Kolbe first identify the structure of salicylate and in collaboration with Lautemann, carry out the first purified synthesis of salicylate in 1859 (Fig. 1)? This was improved upon by his student, Kolbe, some 15 years later who designed the first commercially viable industrial synthesis of salicylate. Serendipitously, MacLagan's paper appeared in the *Lancet* (1876) setting the clinical stage for salicylate's appearance as the first anti-inflammatory pharmaceutical for widespread application. This coincided with the first clinical trial in Berlin, Germany, demonstrating salicylate's effectiveness in treating the symptoms of rheumatoid arthritis. Salicylate's bitter taste and gastric irritation soon caused problems that were solved in 1897 by Felix Hoffman, working for Bayer, who acetylated salicylate leading to the birth of aspirin [1].

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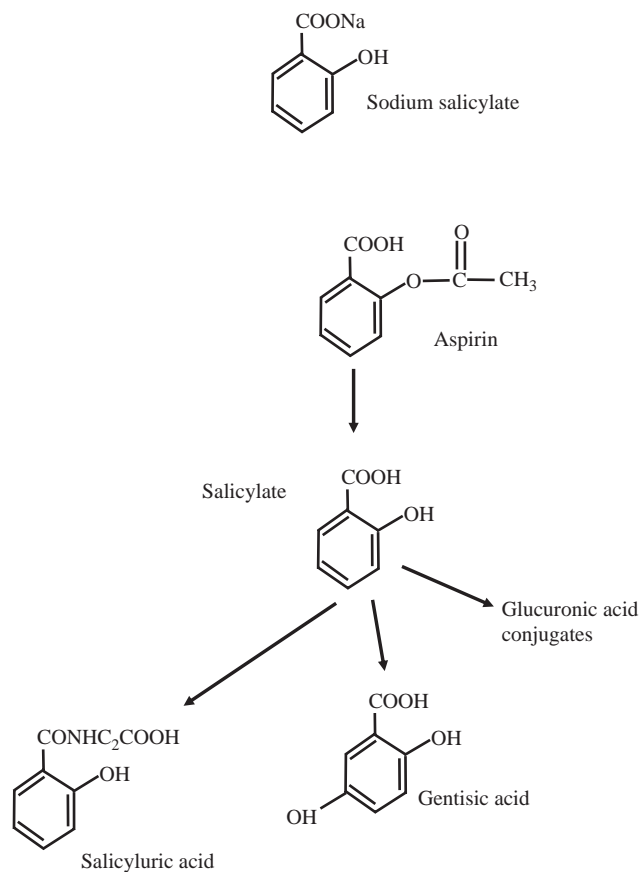


Fig. 1. Pathway of aspirin metabolism in vivo. Following oral administration, aspirin is rapidly absorbed and subjected to first-pass metabolism to form salicylates with plasma half-life in the order of 15–20 min, being detected before salicylate levels are measurable. There are five pathways that account for the clearance of salicylate and therefore aspirin with all metabolites being excreted via the kidney. The metabolites of salicylate are salicyluric acid, glucuronic acid conjugates of salicylic acid (salicyl phenolic glucuronide and salicyl acyl glucuronide) and gentisic acid. Gentisic acid may be formed from gentisic acid via glycine conjugation or from salicyluric acid via microsomal oxidation.

The scope of this mini-review does not allow for the complete testimonial that aspirin's colourful history deserves: from the usage of salicylate in antiquity for inflammation and pain to the countless indications of acetylated salicylate today including cardiovascular diseases, stroke, pregnancy complications, cancer, diabetes, Alzheimer's disease, etc. But, just like the efforts made throughout the 1800s to elucidate the structure of salicylate and find an alternative which culminated in aspirin, huge efforts were being made through the 1900s to understand how aspirin works. In this short review, some of the earlier theories on aspirin's actions will be recounted leading to Sir John Vane's ground-breaking discovery of prostaglandin (PG) inhibition. Consequently, the questions surrounding the PG inhibition theory will be discussed as will the alternative hypotheses proposed to solve the aspirin enigma culminating in the comparatively recent discovery of aspirin-triggered

epi-Lipoxins (epi-LXs), their role in inflammation, and how they may ultimately explain aspirin's anti-inflammatory properties.

2. Aspirin and innate immunity

Aspirin enjoyed worldwide success for over 70 years without a known mechanism of action, confounded, not least, by the fact that little was known about the soluble mediators that initiated and propagated the inflammatory response. Certainly, there was no shortage of ideas by the late 1960s and early 1970s as to how aspirin inhibits acute inflammation, including that aspirin-like drugs uncoupled oxidative phosphorylation [2], inhibited dehydrogenase enzymes dependent upon pyridine nucleotides [3], as well as inhibited some amino transferases and decarboxylases [4]. One of the most promising suggestions at the time was that aspirin and the salicylates could inhibit proteases found at inflammatory sites and which were found to cause tissue damage associated with chronic inflammatory diseases. The major inconsistency with all these theories was that the level of aspirin required to inhibit these enzymes was much higher than the plasma level achieved by anti-inflammatory doses of aspirin and that inhibition of some or even all of these enzymic pathways could not explain aspirin's anti-inflammatory, anti-pyretic and analgesic properties. Thus, a convincing and consistent mechanism was lacking.

Undoubtedly, the first step in unraveling the aspirin/PG story came from the work of Collier and colleagues who crucially identified that aspirin inhibited the bronchoconstriction elicited by bradykinin [5] or the slow-reacting substances of anaphylaxis [6]. One of his graduate students, Priscilla Piper, went to work with Sir John Vane at the Royal College of Surgeons to further understand how aspirin exerted its protective effect against bronchoconstriction. During the course of their studies they identified a number of novel factors that were released during anaphylaxis, including PGE₂, PGF₂α, and what was called at the time rabbit aorta contracting substance, subsequently identified as thromboxane A₂ [1] (Fig. 2). This introduced the first hint of an eicosanoid link in the aspirin story. Vane and colleagues went on to show that aspirin could inhibit thromboxane and PG synthesis in a number of tissues from various species in response to a vast array of stimuli. Hindsight allows us now to see the obvious connection between aspirin and the inhibition of PGs, though what was not known at the time was how PGs were made. It was clear that there was a certain level of PGs constitutively present in most tissues and Vane noticed that their levels increased in response to their various stimulating protocols and concluded that PG synthesis could be enhanced. Vane, admitting that the

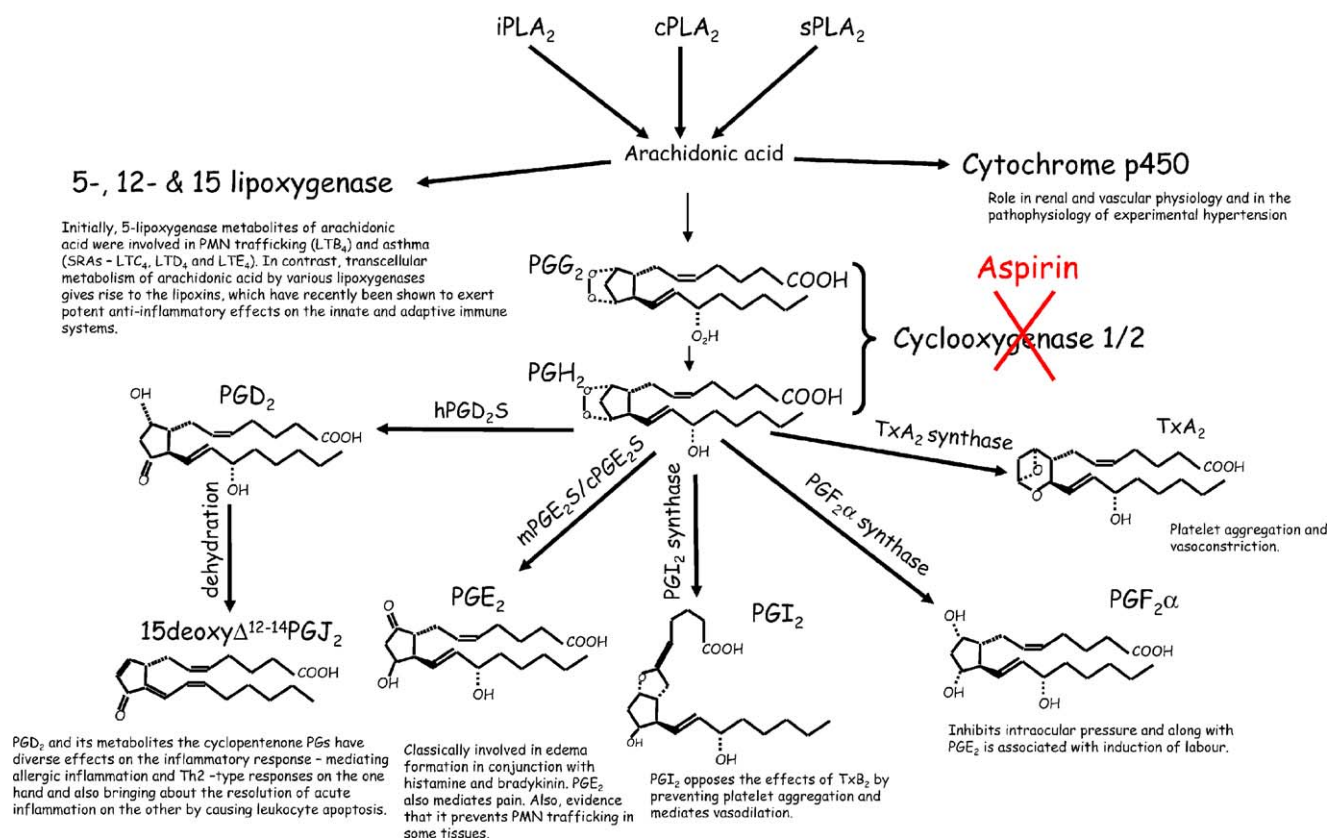


Fig. 2. Arachidonic metabolism by COX, as well as the LOX and cytochrome p450 family of enzymes. Arachidonic acid is released by either cytosolic, secretory or calcium-independent phospholipase A₂. The phospholipase A₂ involved is cell-type and stimulus specific. Liberated arachidonic acid is metabolised by COX 1 or COX 2 first to PGG₂ and then PGH₂. PGH₂ serves as a substrate for a series of down-stream synthases to give rise to the PGS. For PGE₂ synthesis, for instance, PGH₂ is metabolised by constitutive or inducible prostaglandin E₂ synthase. Alternatively, arachidonic acid may be metabolised by the lipoxygenase or cytochrome p450 family of isoenzymes.

wood was not immediately apparent from the trees, suggested that aspirin must be inhibiting the enzyme system that synthesises PGs. And so it did. In 1971 Vane demonstrated that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) dose-dependently inhibited the ability of guinea pig lung homogenates to make PGs. Work by Smith and Willis corroborated these findings in the same issue of *Nature* by showing that aspirin and other NSAIDs inhibited PG synthesis from platelets, the importance of which was reinforced by the fact that not only were these studies done on guinea pig lungs but that aspirin was effective in this regard after oral administration in man.

2.1. Inconsistencies in the prostaglandin theory

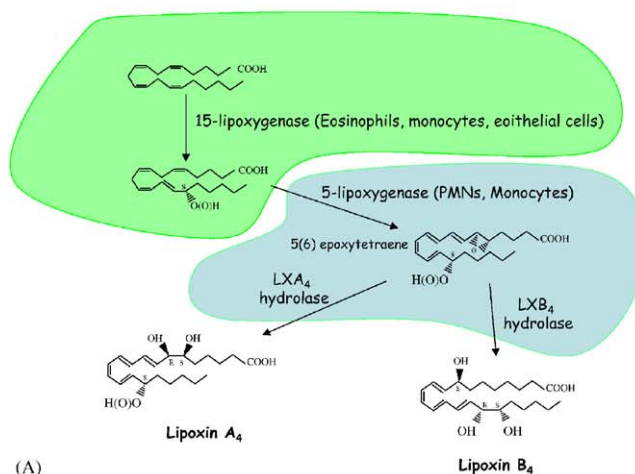
And so it seemed the enigma was solved and all was well in the field of inflammation research. This was so until a number of questions were raised about aspirin's differential effects on PG synthesis and, in particular, the inherent role of PGs in inflammation. Aspirin is unique among the established NSAIDs in that it has effects on both the inflammatory response and cardiovascular system. For instance, it protects against platelet

aggregation in humans at 75 mg [7] whereas 1 g, in man, is required to inhibit acute inflammation [8]. Its protective effects against platelet aggregation are due to the inhibition of thromboxane A₂. Thus, aspirin can inhibit PG synthesis at doses that are considerably lower than that which inhibits inflammation, suggesting that at the higher doses of 1 g aspirin must be inhibiting, inducing, or otherwise altering other pathways pertinent to the inflammatory response in humans besides arachidonic acid metabolism. Another, more convincing argument relating to the aspirin/PG theory lies in the fundamental properties of the PGs. In general, the PGs, acting in synergy with other mediators such as histamine and bradykinin, play a role in oedema formation and contribute to pain but are not generally considered to be chemoattractant for leukocytes [9]. Within the eicosanoid family, this is the responsibility of the leukotrienes, in particular leukotriene B₄, a metabolite of the 5-lipoxygenase pathway of arachidonic metabolism [9]. Therefore, how can the anti-leukocyte accumulating properties of aspirin be explained by inhibiting PGs if such fatty acid hormones play little role in cell trafficking? Moreover, where does this leave aspirin and its true mode of action?

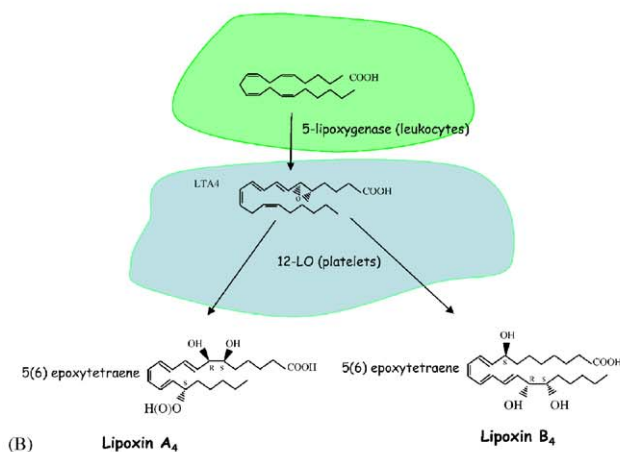
2.2. Aspirin, its other effects and the epi-LXs

Since the 1970s, there have been as many, if not more, theories to explain aspirin's anti-inflammatory properties as there were before Vane discoveries. Of these, it was suggested, for example, that aspirin inhibits NF- κ B activation [10], mediates the release of adenosine [11], activates heat shock proteins [12] and down-regulates inducible cyclooxygenase (COX 2) expression [13], to name but a few. Perhaps one of the most exciting aspects of the mechanism of action of aspirin stems from its ability to trigger LX synthesis (so-called aspirin-triggered epi-LXs) as a result of acetylating the active site of COX 2 in endothelial or epithelial cells, a property not shared with other NSAIDs. This results not in the inhibition of COX 2 enzyme activity, as might be expected, but in the conversion of arachidonic acid to (15 *R*) hydroxyeicosatetraenoic acid (15*R*-HETE), which is rapidly metabolised in a transcellular manner by leukocyte 5-lipoxygenase to 15 epi-LX A₄ or B₄ [14]. There are two other pathways by which LXs may be generated and although catalogued in greater detail elsewhere in this issue, it is relevant to mention them here. Importantly, these alternative pathways are not generated by aspirin or any other NSAID. Briefly, the second pathway involves the insertion of O₂ to the OH group on carbon 15 of arachidonic acid by 15-lipoxygenase within eosinophils, monocytes, or epithelia cells. Following its release from these cells and entry to either PMNs or monocytes, a 5,6-epoxytetraene is generated by 5-lipoxygenase, which is then hydrolysed within these recipient cells by either LXA₄ hydrolase or LXB₄ hydrolase to bioactive LX A₄ and B₄, respectively [15]. The third route of LX biosynthesis results from the generation of LTA₄ by 5-lipoxygenase within leukocytes, its release and subsequent uptake by platelets and metabolism by 12-lipoxygenase to LXA₄ and B₄ [16,17], Fig. 3A–C. The three major pathways described above can operate independently or simultaneously within the vasculature. GM-CSF primed neutrophils, for example,

Lipoxin biosynthesis - Pathway 1



Lipoxin biosynthesis - Pathway 2



Aspirin-triggered epi-lipoxin synthesis - Pathway 3

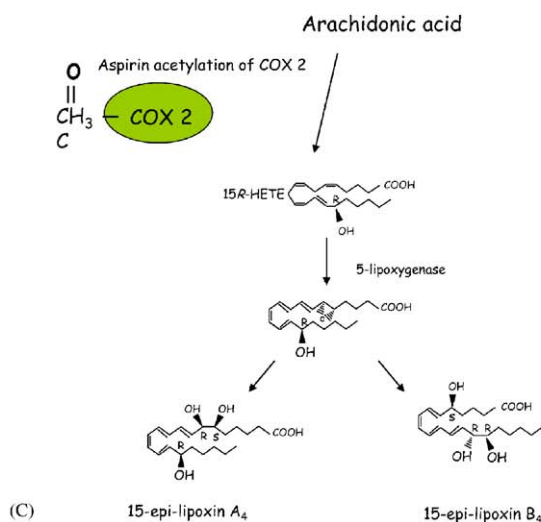


Fig. 3. Biosynthesis of LXs occurs through three distinct biosynthetic pathways. (A) The first is via the action of monocytes or epithelial cell 15-LOX. Molecular oxygen is inserted into carbon 15 of arachidonic acid yielding 15(*S*) hydroperoxyeicosatetraenoic acid, which can then serve as a substrate for PMN 5-LOX to generate 5,6-epoxytetraene. This unstable epoxide intermediate is converted to LXA₄ and LXB₄ by epoxide hydrolases in leukocytes. The second route of (B) platelet–PMN interaction, 5-LOX in PMNs metabolises arachidonic acid to leukotriene A₄, which is taken up by platelets in a trans-cellular manner and converted by platelet 12-LOX to LXA₄. A final and unorthodox route for LX metabolism involves aspirin. (C) Aspirin acetylates the active site of COX 2 resulting not in the inhibition of COX 2 enzyme activity but the conversion of arachidonic acid to 15(*R*)-hydroxyeicosatetraenoic, which when released from endothelial and epithelial cells may be transformed by leukocyte 5-LOX to generate 15 epi-LXA₄ or 15 epi-LXB₄.

recruited to the inflammatory site can interact with platelets. After platelets adhere to the neutrophil surface, active PMNs generate LTA_4 , which is released and transformed by platelet 12-lipoxygenase to generate LXs. Within the vasculature, the aspirin-triggered LX pathway can also be initiated when activated endothelial cells interact with adherent neutrophils to generate 15 epi-LXs. Leukocytes interacting with epithelial cell surfaces, as in the case of respiratory, renal, or gastrointestinal inflammation, can also generate LXs through bi-directional routes in which (15*S*)-HETE and (15*R*)-HETE are released by epithelial cells and converted to LXs by neutrophils. The other component of this bi-directional interaction can involve neutrophil-released LTA_4 , which is converted by 15-lipoxygenase in epithelial cells, in particular tracheal epithelial cells, to generate LXs.

2.3. Bio-protective properties of LXs

It has been well characterised by now that aspirin can trigger 15 epi-LXA₄ synthesis in experimental models of acute inflammation [18] and tissue injury [19] as well as in a clinical setting [20,21]. Aspirin is known to increase plasma [21] and urinary [20] levels of 15 epi-LXA₄ in healthy human volunteers as well as in rodents. In a recent study it has been shown that aspirin, consumed once daily for 8 weeks by healthy human volunteers at doses of 81 mg/kg, resulted in a significant increase in plasma 15 epi-LXA₄ synthesis with doses of 325 mg/kg bringing about a borderline increase and 650 mg/kg having no significant effect [21]. These data are of potential importance because recent reports have suggested that doses of aspirin that exceed 1 g/day do not confer any greater protection against adverse vascular events than that attained with lower doses of aspirin, typically 75–150 mg/day [22,23]. Therefore, given the role of inflammation in cardiovascular disease and endothelial dysfunction and the anti-inflammatory properties of the epi-LXs, it is possible that the local generation of 15 epi-LXA₄ within the leukocyte/endothelial cell interface is of great importance in aspirin-mediated cardio-protection.

Staying with this theme of vascular reactivity, LXA₄ and B₄ promote vasorelaxation and relax aorta and pulmonary arteries [24]. LXA₄ also reverses pre-contraction of the pulmonary artery induced by PGF_{2α} and endothelin 1. The mechanisms of LXA₄- and LXB₄-induced vasodilation involve endothelium-dependent vasorelaxation as well as PG-dependent and -independent pathways. In some tissues, LXs can stimulate the synthesis of prostacyclin [25] and nitric oxide (NO) by endothelial cells [26], both of which mediate vasodilation and which may play an important part in the anti-inflammatory properties of the aspirin-triggered epi-LXs, as discussed later. Due to the very short half-lives

of the LXs, a range of stable biologically active analogues have been designed and their anti-inflammatory effects demonstrated in various experimental animal models of acute inflammation of diverse aetiologies. Specifically, LXs and epi-LXs display selective pro-resolution actions on leukocytes that include inhibition of PMN chemotaxis [27], PMN adhesion to and transmigration through endothelial cells [28], as well as PMN-mediated increases in vascular permeability [29]. Their actions are as complex as the LXs and also stimulate monocyte chemotaxis and adherence [30,31], although without causing degranulation or release of reactive oxygen species [32]. In addition, LXs and their stable analogues stimulate non-phlogistic phagocytosis of apoptotic PMNs by monocyte-derived macrophages [33,34], a critical event in inflammation that signals resolution and paves the way for a return to tissue normality.

These studies on LX metabolism are uncovering surprising new avenues in anti-inflammation research, putting fatty acid metabolites at the forefront of potential drug therapy. These studies are also challenging existing dogma that not all eicosanoids are detrimental to inflammation, as previously thought, and are putting a balanced view of their role in pathophysiology. In support of this notion is the recent and very surprising paper showing that eicosanoids of the LXs family are orally active in models of acute inflammation [35]. Also of great interest is the finding that a single dose of 1 μg of aspirin-triggered epi-LXA₄ conferred significant protection against the clinical symptoms of experimental graft-versus-host disease including skin lesions, eye inflammation, diarrhoea, weight loss and importantly almost doubled life-expectancy [36]. Clearly, LXs represent endogenous factors with counter-regulatory effects on the innate and adaptive immune systems that may provide the basis for new and exciting drug discovery.

2.4. 15 epi-LXA₄ and nitric-mediated anti-inflammatory properties of aspirin

The theme of cross-talk between the inflammatory response and the cardiovascular system continues here with the observation that aspirin can trigger large amounts of peripheral blood NO through its ability to elicit 15 epi-LXA₄ synthesis [18]. In a series of experiments using rodent acute pleuritis or peritonitis models, a single anti-inflammatory dose of aspirin resulted in high levels of nitrite/nitrate appearing in the peripheral blood of these animals after a few hours, which correlated inversely with a dose-dependent and significant reduction in leukocyte accumulation at the inflammatory site (Fig. 4). This was in contrast to other NSAIDs such as salicylate, piroxicam and indomethacin which had no effect on plasma NO. This observation

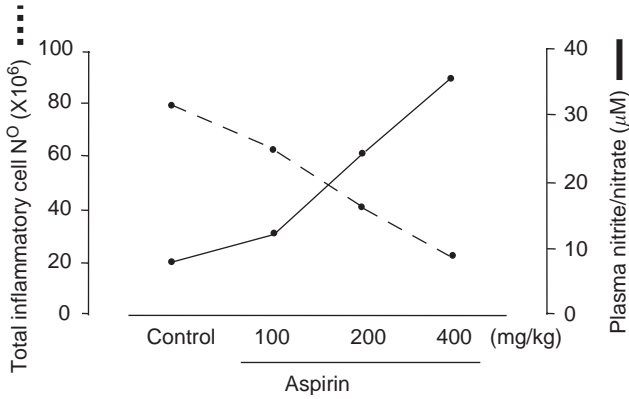


Fig. 4. The effects of aspirin on NO-synthesis in the peripheral blood of rats bearing a carrageenin-induced pleurisy. Aspirin was dosed orally 1h before the intrapleural injection of carrageenin and inflammation (inflammatory cell numbers) and peripheral blood sampled 4h after stimulus for levels of nitrite/nitrate by the nitrate reductase–Griess reaction. Essentially, aspirin reduced leukocyte trafficking into the pleural cavity, as expected, but also brought about a significant and dose-dependent increase in plasma NO.

knockout mice with IL-1β-induced peritonitis [18]. We managed to show that aspirin generation of NO was most likely through its triggering of 15 epi-LXA₄. Aspirin and 15 epi-LXA₄ were shown to inhibit leukocyte trafficking in an NO-dependent manner using intravital microscopy on IL-1β-stimulated mouse mesentery. Not only did aspirin inhibit leukocyte–endothelial interaction in a manner similar to NO in wild-type mice but both aspirin and 15 epi-LXA₄ had markedly reduced effects on leukocyte–endothelial cell adherence in eNOS- and iNOS-deficient mice compared with wild type. From these findings we concluded that once ingested, aspirin is exposed to the vasculature where it acetylates COX 2 within the endothelium or circulating leukocytes to trigger 15 epi-LXA₄ which, in turn, elicits NO synthesis from both eNOS and iNOS. Ultimately, aspirin-triggered NO mediates the anti-inflammatory effects of aspirin in the microcirculation by negatively regulating leukocyte/endothelium interaction (Fig. 5).

2.5. What has aspirin told us about the inflammatory response?

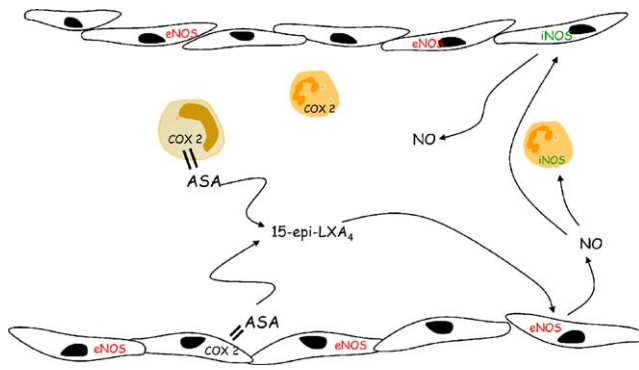


Fig. 5. Hypothesis of the events occurring in the microvasculature after ingestion of aspirin. Once in the systemic circulation, aspirin acetylates constitutively expressed COX 2 in the microvascular endothelium and/or the circulating leukocytes to generate 15 epi-lipoxin A₄. This 15 epi-lipoxin A₄ then triggers eNOS-derived NO synthesis. It is hypothesised that this eNOS-derived NO plays two roles—the control of early phase leukocyte/endothelial cell interaction (up to 2h) and the subsequent induction of iNOS in the endothelium, leukocytes or both. NO from iNOS is then responsible for the later phase of control of leukocyte trafficking (4/6h and beyond).

In addition to showing how aspirin works in murine peritonitis, these studies have revealed a great deal of additional information about the onset phase of acute inflammation. First, it tells us that in addition to aspirin, the epi-LXs also regulate cell trafficking through NO. Secondly, and without going into great detail, it seems from the published data [18] that both eNOS and iNOS are needed to control leukocyte trafficking during acute inflammation and that eNOS is required to signal subsequent iNOS expression. In terms of the regulation of NO synthesis during acute inflammation, how do aspirin and the LXs increase NO synthesis and what cross-talk exists between eNOS and iNOS for the regulation of leukocyte trafficking in aspirin-treated inflammation? The former is difficult to address at this stage. However, regarding the latter, it is known that NO has a biphasic effect on NF-κB activity in murine macrophages, being able to up- and down-regulate the expression of a number of pro-inflammatory proteins, including iNOS [38]. It appears that low levels of NO, as produced from eNOS, activates NF-κB whereas higher levels of NO, typical of iNOS, inhibits NF-κB. In support of this, it was recently shown that lipopolysaccharide-stimulated bone marrow derived macrophages from eNOS knockout mice show greatly reduced NF-κB activity and iNOS expression compared to wild-type cells [39]. These authors demonstrated that eNOS triggered iNOS expression, in part, through soluble guanylate cyclase (sGC) as enhanced iNOS expression, brought about by levels of NO similar to that produced by eNOS, were inhibited by ODQ (a sGC inhibitor) but increased by BAY 41-2272 (sGC activator). As NO can activate NF-κB in a sGC-dependent manner [38], the potentiating

was exciting for two reasons. First, NO has profound protective effects on the cardiovascular system and could help explain some of the effects of the LXs detailed above and second, it inhibits leukocyte trafficking [37]. It is NO's ability to prevent leukocyte/endothelial cell interaction during acute inflammation that interested us most. After a series of experiments we found that inhibiting aspirin-elicited NO pharmacologically nullified the anti-inflammatory effects of aspirin. Moreover, aspirin was not anti-inflammatory in either constitutive (eNOS) or inducible NO synthase (iNOS)

effects of NO on NF- κ B activity and subsequent iNOS expression in macrophages are likely to occur, at least in part, via activation of sGC and the production of cGMP. A critical role for this NO/cGMP pathway in cytokine signaling of iNOS expression was also shown in human mesangial cells [40]. Thus, inhibiting the first wave of NO stimulated by IL-1 β or tumour necrosis factor with L-NAME resulted in a subsequent reduction in iNOS expression and activity, which was reversed using an NO donor. Induction of iNOS was shown to be sGC dependent as ODQ reduced cytokine and NO donor-stimulated iNOS expression, effects that were rescued with a cell-permeable cGMP analogue. Collectively, these studies suggest that if the initial NO-mediated response to stimulation is absent, the magnitude of subsequent iNOS expression is diminished. These in vitro findings may explain why plasma NO levels in aspirin-treated eNOS^{-/-} and iNOS^{-/-} mice are much lower than aspirin-treated wild-type animals [18]. In the absence of eNOS, for instance, iNOS expression maybe reduced producing correspondingly low amounts of NO. Similarly, in the absence of iNOS, though eNOS activity maybe enhanced slightly by aspirin, levels of NO from this constitutive isoform will also be low. While there is a possible contribution of NO from nNOS in this setting, based on the evidence so far it appears that in acute inflammation NO mediates the anti-inflammatory effects of aspirin and LXs and that this NO comes from the initial activation of eNOS, which subsequently triggers iNOS expression. Thus, activation of eNOS may be central to the anti-inflammatory actions of aspirin and the LXs.

In addition to animals bearing a localised inflammation, we found that aspirin and 15 epi-LXA₄ also elicited NO synthesis in naïve animals, i.e. mice without any systemic or localised inflammatory response [18]. This suggests that the biochemical machinery required to manufacture NO in response to aspirin is constitutively expressed. That 15 epi-LXA₄ mediates aspirin-generated NO identifies COX 2/5-lipoxygenase as this constitutively expressed pathway. COX 2 is not classically thought of as a constitutively expressed enzyme. However, given the size of the vasculature it has been proposed that the vascular endothelium may contain focal regions or “hot spots” under stress that express COX 2 and can generate substantial amounts of COX 2-derived epi-LXs with aspirin treatment [41]. Certainly, it was found that a physiological level of steady laminar shear stress, an in vitro model that mimics the average wall shear encountered in the vasculature, caused a sustained expression of endothelial COX 2 [42]. This constitutive expression of COX 2 helps to explain why we found increased plasma NO in naïve, un-inflamed animals. In addition to the endothelium, COX 2 could also be constitutively expressed in circulating leukocytes. Indeed, we found that about 10% of circulating PMNs from naïve rats constitutively express COX 2

[43]. However, whether it is in the endothelium or circulation leukocytes, the exact cellular source of constitutively expressed COX 2 remains to be identified.

3. Concluding remarks

Aspirin has never ceased to amaze the clinician, the scientist, and society as a whole. Not only does it present with new benefits for treating an ever-expanding list of apparently unrelated diseases at an astounding rate but it also enhances our understanding of the nature of these disease processes. Originally, the beneficial effects of aspirin were shown to stem from its inhibition of PG synthesis. However, in addition to inhibiting COX activity, aspirin can also inhibit pro-inflammatory signaling pathways, gene expression, and other factors distinct from eicosanoid biosynthesis that drive inflammation as well as enhance the synthesis of endogenous protective anti-inflammatory factors. It is somehow felt that its true mechanism of action in anti-inflammation remains unclear. In this mini-review, data from a series of experiments proposing that one of aspirin’s predominant roles in inflammation is the induction of epi-LX and NO biosynthesis, was discussed. It was argued that this NO-inducing effect is exclusive to aspirin due to its unique ability, among the family of traditional anti-inflammatory drugs, to acetylate the active site of inducible COX 2 and generate epi-LXs. Whether 15 epi-LXA₄’s triggering of protective NO represents the final few tricks in aspirin’s repertoire of anti-inflammatory effects is yet to be determined. However, given its history of revealing new and unlikely properties, it is fair to suspect that it may still have something new and profound to reveal. As these epi-LXs are increasingly shown to have profound roles in a range of host defense responses, perhaps the important question now should be “is the true mechanism of action of aspirin not due to the inhibition of putative pro-inflammatory mediators, but the induction of anti-inflammatory ones?”

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