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Effects of Low-Dose Aspirin on Acute Inflammatory Responses in Humans

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Aspirin is a unique nonsteroidal anti-inflammatory drug; at high doses (aspirin\textsuperscript{high}, 1g), it is anti-inflammatory stemming from the inhibition of cyclooxygenase and proinflammatory signaling pathways including NF-κB, but is cardioprotective at lower doses (aspirin\textsuperscript{low}, 75 mg). The latter arises from the inhibition of thromboxane (Tx) B\textsubscript{2}, a prothrombotic eicosanoid also implicated in polymorphonuclear leukocyte trafficking. As a result, aspirin\textsuperscript{low} is widely used as a primary and secondary preventative against vascular disease. Despite this and its ability to synthesize proresolving 15-epi-lipoxin A\textsubscript{4} it is not known whether aspirin\textsuperscript{low} is anti-inflammatory in humans. To address this, we generated skin blisters by topically applying cantharidin on the forearm of healthy male volunteers, causing an acute inflammatory response including dermal edema formation and leukocyte trafficking. Although not affecting blister fluid volume, aspirin\textsuperscript{low} (75 mg, oral, once daily/10 days) reduced polymorphonuclear leukocyte and macrophage accumulation independent of NF-κB-regulated gene expression and inhibition of conventional prostanoids. However, aspirin\textsuperscript{low} triggered 15-epi-lipoxin A\textsubscript{4} synthesis and up-regulated its receptor (FPRL1, ALX). From complimentary in vitro experiments, we propose that 15-epi-lipoxin A\textsubscript{4} exerts its protective effects by triggering antiadhesive NO, thereby dampening leukocyte/endothelial cell interaction and subsequent extravascular leukocyte migration. Since similar findings were obtained from murine zymosan-induced peritonitis, we suggest that aspirin\textsuperscript{low} possesses the ability to inhibit mammalian innate immune-mediated responses. This highlights 15-epi-lipoxin A\textsubscript{4} as a novel anti-inflammatory working through a defined receptor and suggests that mimicking its mode of action represents a new approach to treating inflammation-driven diseases. The Journal of Immunology, 2009, 183: 2089–2096.

In our attempts to treat inflammation-mediated diseases, drugs were developed to target pathways believed to drive inflammation, most notably the nonsteroidal anti-inflammatory drugs (NSAIDs)\textsuperscript{3} (1). Aspirin is the archetypal NSAID found to inhibit the cyclooxygenase pathway of arachidonic acid metabolism (2, 3) being anti-inflammatory at 1g (aspirin\textsuperscript{high}) (4), but cardioprotective at lower doses (75–150 mg/day, aspirin\textsuperscript{low}) through the inhibition of platelet-derived thromboxane (Tx) A\textsubscript{2} (5, 6). The importance of aspirin therapy in this setting was underscored by a meta-analysis from the Antiithrombotic Trialists’ Collaboration (287 randomized trials of antplatelet therapy in patients with high risk of occlusive vascular events) that demonstrated a 32% reduction in nonfatal myocardial infarction, nonfatal stroke, and vascular death in patients treated with aspirin\textsuperscript{low} at 75–150 mg daily (7).

Aspirin\textsuperscript{low} also inhibits pathways inherent to innate immunity including the production of Tx A\textsubscript{2} (8), which is suggested to facilitate the polymorphonuclear leukocyte (PMN)-platelet interaction that leads to PMN transmigration into inflamed tissues (9, 10). Moreover, aspirin\textsuperscript{low} triggers the synthesis of novel lipid metabolites that directly halt leukocyte trafficking and elicit proresolving effects (11). In addition, there is evidence that aspirin down-regulates proinflammatory signaling pathways including NF-κB (12, 13). This suggests that aspirin at levels used in cardioprotection may be anti-inflammatory. Despite this, there is no direct evidence that aspirin\textsuperscript{low} alters the course of acute inflammation or triggers the early resolution of innate immune-mediated responses in humans.

In a well-characterized model of cantharidin-induced acute inflammation (14–17), 75 mg of aspirin, dosed orally for 10 days, reduced PMN and macrophage accumulation. Its protective mechanism is dependent on 15-epi-lipoxin A\textsubscript{4} synthesis signaling through its receptor (FPRL1 or ALX) (18). 15-Epi-lipoxin A\textsubscript{4}, in turn, triggers anti-adhesive NO release which negatively regulates leukocyte/endothelial interaction; a critical determinant in extravascular leukocyte accumulation, showing for the first time that aspirin\textsuperscript{low} is anti-inflammatory in humans.

Materials and Methods

Inflammatory models and drug treatment

Two blisters were elicited on the ventral aspect of the forearms of male healthy volunteers as previously described (14–17) by applying 10 μl of
0.1% cantharone (Dormer Labs). Aspirinlow (75 mg) was taken daily for 10 days before eliciting a second set of blisters on the contralateral forearm with aspirin consumed for the duration of the response up to 72 h. Aspirinhigh (1g) was administered in three doses of 300 mg every 8 h.

**FIGURE 1.** Aspirinlow is anti-inflammatory in humans. Blisters were elicited on the forearms of male healthy volunteers in response to cantharidin to establish baseline responses. Aspirinlow (75 mg) was then consumed for 10 days followed by two additional blisters on the contralateral arm, with aspirin consumption maintained for the duration of the blister. Thus, each volunteer acted as his own control (ctl). Aspirinlow significantly reduced total leukocyte accumulation (A) as well as PMNs (B) and macrophages (C). Conventional high-dose aspirin (aspirinhigh, 1g) exerted a similar anti-inflammatory effect in this model on total cells (D), PMNs (E), and macrophages (F). G. Moreover, blisters repeated on five volunteers without aspirin 1 wk apart demonstrated equivalent cell accumulation, suggesting that the effects observed were not due to desensitization or conditioning to cantharidin. Data are expressed as mean ± SEM, *p ≤ 0.05 and ***, p ≤ 0.001.

**FIGURE 2.** Effects of aspirinlow on plasma and blister arachidonic metabolites. To elucidate aspirinlow protective effects in humans, plasma and blister levels of COX metabolites of arachidonic acid were measured before and after aspirinlow. A, Blister PGE2 was inhibited by aspirinlow and (B) aspirinhigh with aspirinlow also reducing (C) plasma and (D) blister TxB2 (stable metabolite of prothrombotic TxA2). In addition to being prothrombotic, Tx also facilitates PMN trafficking via platelet interaction to sites of injury (9, 10). Despite this, mice bearing a zymosan-induced peritonitis and dosed chronically with aspirin at doses (0.2 mg/kg) that (E) significantly reduces TxA2 showed no reduction in (F) peritoneal PMN numbers. No effects on inflammation were found with (G) chronic salicylate, indicating an aspirin-specific effect. Finally, PGI2 (measured as 6-keto-PGF1α) in plasma (H) and blister fluid (I) was reduced by aspirinlow, which despite causing edema formation in rodents (22), did not correlate with blister edema levels (J). Data are expressed as mean ± SEM, *p ≤ 0.05 and ***, p ≤ 0.01. ctl, Control.
24 h before blistering. Ethical approval was obtained from University College London Ethics (Ethics Project Identification No. 1309/001). For murine peritonitis, all animals (C57BL/6J) were bred under standard conditions and maintained in a 12-h/12-h light/dark cycle at 22 ± 1°C and given food and tap water ad libitum in accordance with United Kingdom Home Office regulations. Peritonitis was induced by the i.p. injection of 1 mg of type A zymosan (Sigma-Aldrich) with the effects of chronic aspirin (either 0.2, 2.0, or 200 mg/kg, once daily for 10 days) on leukocyte numbers determined 4 h later. For human blister and murine peritonitis, cells were enumerated by hemocytometer.

FACS, lipid mediators, and NO

Anti-CD14, CD16B, and FPRL1 (human) along with LY6G (murine anti-PMN marker) with isotype controls were from Serotec or BD Biosciences. Peripheral blood and blister-derived leukocytes were acquired by FACS Calibur (BD Biosciences) using appropriate compensation where necessary and data were analyzed by CellQuest Pro. PGE₂, TxA₂, and nitric oxide were measured by enzyme immunoassay (GE Healthcare), while 15-epi-lipoxin A₄ was measured by ELISA (Neogen). NO was measured as total nitrite and nitrate in samples deproteinated by ultracentrifugation followed by analysis using the nitrate reductase assay followed by Griess reaction and confirmed by chemiluminescence. Cytokines and chemokines were measured initially by Multiplex cytokine array analysis (Bio-Rad) using the manufacturer’s protocols.

Leukocyte isolation, in vitro cell culturing, and treatment protocols

For determining 15-epi-lipoxin A₄ production (55,6R,15R-trihydroxy-eicosa-7E,9E,11Z,13E-tetraenoic acid) production from PMN-HUVEC interaction, confluent HUVECs (passages two to four) were stimulated with IL-1β (1 ng/ml) for 24 h, washed in HBSS, and treated with 30 μM aspirin or vehicle (0.1% DMSO) for 20 min at 37°C and 20 μM arachidonic acid for a further 60 s. Thereafter, each was incubated with fresh isolated PMN (HUVECs:PMN cell ratio = 1:10) followed by costimulation with 5 μM ionophore A23187 for 30 min at 37°C. For determinations of leukocyte adherence to HUVECs, PMN were treated with vehicle or 10 nM 15-epi-lipoxin A₄ for 30 min at 37°C and washed once before coincubation with HUVECs and 100 nM leukotriene B₄. Three hours later, plates were washed twice to remove nonadhered PMNs with the amount of adhered PMN determined by the quantity of myeloperoxidase per well. For 15-epi-lipoxin A₄ treatment of HUVECs, monocytes, and macrophages, 0.003–0.3 μM was used along with LPS (1 μg/ml) and BOC-2 (1 μM). Human monocytes were isolated from Eloha (to sediment RBC)-treated blood from healthy male volunteers followed by density gradient centrifugation using Percoll and incubated for 4 days with GM-CSF (100 ng/ml) in RPMI 1640 medium to differentiate to macrophages.

Statistical analysis

Data were analyzed using the two-tailed paired Student t test for normally distributed data, with p < 0.05 being considered significant. A Wilcoxon–matched pairs test was used to analyze data standardized to the number of cells accumulated within the blister (15-epi-lipoxin A₄/10⁶ cells); because it did not follow a Gaussian distribution, p < 0.05 were considered significant. When comparing two groups with unequal variances, a two-tailed unpaired t test with the Welch correction was used. For animal studies and in vitro assays, data were analyzed by ANOVA followed by followed by the Bonferroni t test.

Results

Chronic aspirinlow inhibits inflammatory cell recruitment

Cantharidin, a vesicant from the hemolymph of blister beetles (Meloidae coleopteran) causes acantholysis and blister formation upon contact with skin and is characterized by dermal perivascular leukocyte infiltration (14–17). Using cantharidin, blisters were established on the ventral aspect of the forearm of 26 healthy male volunteers whose baseline inflammatory response (without aspirin treatment) was determined at 24 h (onset) and 72 h (resolution; Fig. 1, A–C). Total inflammatory cells, including PMNs and macrophages, were maximal at 24 h with both cell types declining in number by 72 h. Thus, cantharidin-induced skin blistering results in an acute inflammatory infiltrate that resolves over time. To examine the effects of aspirinlow in this model, each volunteer orally consumed 75 mg once daily for 10 days and had two additional blisters elicited on the contralateral forearm, each subject therefore acted as his own control. Blisters were repeated 1 wk after baseline responses were determined with aspirinlow consumption continuing for the duration of blister formation, i.e., during the inflammatory response up to 72 h. Aspirinlow significantly inhibited total inflammatory cell infiltration, reducing CD16B-positive PMNs and CD14-positive macrophage accumulation by ~65% (Fig. 1, A–C), but had no effect on edema formation (Fig. 2J). For comparison, we also examined the effects of aspirinhigh with a total of 1 g dosed...
over a period of 12 h before blistering, resulting in an expected reduction in inflammation (Fig. 1, I–F). Thus, chronic consumption of daily low-dose aspirin (75 mg) to healthy individuals caused an anti-inflammatory effect in a model of leukocyte trafficking. Blisters repeated on five volunteers without aspirin 1 wk apart demonstrated that the effects we observed were not due to desensitization or conditioning response developing to cantharidin (Fig. 1 G).

Aspirinlow does not work through inhibition of conventional cyclooxygenase (COX) pathways

Traditionally, PG synthesis is inhibited by aspirin (2), thereby explaining the anti-inflammatory properties of NSAIDs. In this present study, both aspirinlow (Fig. 2A) and aspirinhigh (Fig. 2B) lowered blister PGE2. However, although PGE2 along with histamine and bradykinin mediate edema formation, PGE2 is not responsible for mediating leukocyte trafficking (19). Indeed, by signaling via its EP2/4 receptors, PGE2 may repel PMN accumulation by elevating intracellular cAMP. Thus, it is unlikely that the reduction in blister PGE2 is responsible for the concomitant reduction in PMN and macrophage numbers by aspirinlow.

Reduced TxA2 may be an alternative explanation due to the role platelet-leukocyte interaction plays in inflammatory extravascular PMN accumulation (9, 10, 20) and the obligatory role Tx plays in this setting via platelet GPIIb/IIIa (21). However, although aspirinlow inhibited plasma TxA2 (measured as the stable metabolite TxB2, Fig. 2C), it had no effect on blister levels of this prostanoid (Fig. 2D). Moreover, there was no significant correlation between TxA2 and PMN numbers before/after aspirinlow.
after aspirin. Thus, the inhibition of plasma TxA2 by aspirin low does not necessarily explain its anti-inflammatory effects in skin blisters. Taking this further and trying to mimic the experimental protocol in humans, we administered mice for 10 days with a dose of aspirin (0.2 mg/kg) that is not anti-inflammatory in rodents but that diminishes TxA2 (Fig. 2E). This is in contrast to higher levels (2 mg/kg and above) that is anti-inflammatory in mice (see Fig. 6). Despite reduced TxA2 in these animals, there was no concomitant change in PMN numbers in a zymosan-induced murine peritonitis (Fig. 2F). An equivalent treatment regimen with salicylate had no effect on TxA2 or inflammation, suggesting that the effects of aspirin observed in rodents, at least, are caused by aspirin and not its metabolites (Fig. 2G). Thus, although we are not excluding a role for platelets in facilitating PMN trafficking during acute inflammation in humans, these data question the role TxA2 may play in this process. Aspirin low also reduced plasma and blister prostacyclin (PGI2, measured as 6-keto-PGF1α; Fig. 2H) and although PGI2 mediates edema formation in rodents (22), reduced blister 6-keto-PGF1α was not associated with altered edema levels. Therefore, although aspirin low inhibited prostanooid synthesis during acute inflammation in humans, COX inhibition may not be the principle mechanism by which it inhibits cell trafficking. Moreover, some of the properties of COX metabolites established in rodents may not apply to humans.

**Proinflammatory cytokine/chemokine synthesis is not a target for aspirin low**

Aspirin and salicylate inhibit NF-κB (12, 13), albeit at either suprapharmacological levels or at plasma levels found upon ingestion of traditional anti-inflammatory levels of aspirin. However, as the inhibitory effects of cardioprotective aspirin low on proinflammatory signaling pathways is less clear, we measured levels of well-known NF-κB target gene products in blister cell-free inflammatory exudates including IL-8, IL-1β, and MCP-1, all of which were unaltered by aspirin low (Fig. 3, A–F). These data show that in experimental models of acute inflammation in mice, aspirin can inhibit plasma TxA2 at doses that have no effect on PMN accumulation as well as alter leukocyte trafficking without affecting NF-κB-dependent gene expression.

**Aspirin low triggers 15-epi-lipoxin A4 synthesis and ALX expression**

Of all of the NSAIDs, only aspirin possesses the unique ability to trigger lipoxin synthesis (so-called aspirin-triggered epi-lipoxins) as a result of acetylating the active site of COX2 in endothelial cells (23). By acetylating COX2, aspirin causes the enzyme to convert arachidonic acid to (15R)-hydroxyeicosatetraenoic acid (15R-HETE), which is rapidly metabolized in a transcellular manner by leukocyte 5-lipoxygenase to 15-epi-lipoxin A4 or B4. Lipoxins and aspirin-triggered epi-lipoxins signal through two receptors, the aryl hydrocarbon (24) and FPRL1 or ALX receptor (18) and in doing so inhibit PMN-mediated responses by dampening cytokine and chemokine synthesis (25, 26). Aspirin low increased blister levels of 15-epi-lipoxin A4 at 24 h (Fig. 4A) and also increased ALX expression on peripheral blood leukocytes. Specifically, under resting conditions, ALX is constitutively expressed on naive peripheral blood PMNs with equivalent levels of expression on inflamed (blister) PMNs (Fig. 4B). However, aspirin low significantly increased ALX on naïve peripheral blood

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**FIGURE 5.** Aspirin low–triggered 15-epi-lipoxin A4 regulates leukocyte-endothelial interaction in an NO-dependent manner. A, HUVECs were stimulated in vitro with leukotriene B4 for 24 h and incubated with aspirin (10–30 μM) and arachidonic acid (20 μM) to generate aspirin/COX2-derived 15R-HETE, which was taken up by activated PMNs (calcium ionophore 5 μM), converting it to 15-epi-lipoxin A4. B, Adding 15-epi-lipoxin A4 (30 nM) to stimulated HUVEC/PMN cocultures significantly reduced PMN adherence to endothelial cells in a BOC-2 (FPRL1 receptor antagonist, 1 μM) and l-NMMA (100 μM)-dependent manner. Taking these experiments further, 15-epi-lipoxin A4 (0.3 pg/ml to 0.3 μg/ml) elevated NO release from stimulated HUVECs (C) as well as monocytes and macrophages (D) in an l-NMMA and BOC-2-dependent manner. Data are expressed as mean ± SEM. *, p ≤ 0.05 and **, p ≤ 0.01. indo, Indomethacin.
PMNs (Fig. 4C). Unlike PMNs, peripheral blood CD14 ALX-expressing monocytes (Fig. 4D) with aspirinlow increasing its expression on peripheral blood monocytes (Fig. 4E). Nonetheless, despite an increase in 15-epi-lipoxin A4 and its receptor after aspirinlow, there was no corresponding reduction in blister cytokines/chemokines (Fig. 3, A–J). This questions whether aspirinlow exerts its protective effects within the blister.

Aspirinlow modulates leukocyte-endothelial cell interaction

Lipoxins modulate PMN adherence to inflamed mesenteric microvascular endothelial cells in a NO-dependent manner (27). We therefore questioned whether aspirinlow works in the lumens of the microvasculature that serves the inflamed blister by preventing PMN-endothelial cell interaction in a 15-epi-lipoxin A4/NO-dependent source remaining unidentified (data not included). Based on in vitro data where 15-epi-lipoxin A4 elicits NO from resting HUVECs (Fig. 5A), with aspirinlow increasing its expression on peripheral blood monocytes (Fig. 4E). Nonetheless, despite an increase in 15-epi-lipoxin A4 and its receptor after aspirinlow, there was no corresponding reduction in blister cytokines/chemokines (Fig. 3, A–J). This questions whether aspirinlow exerts its protective effects within the blister.

Aspirinlow is also anti-inflammatory in mice

Aspirin was dosed orally at 2 mg/kg once daily for 10 days followed by zymosan (1 mg, i.p.) and an assessment of total PMN numbers was determined 4 h later (A) along with cell-free exudate levels of PGE2 (B) and proinflammatory cytokines (C–E). Data are expressed as mean ± SEM. *p ≤ 0.05 and **p ≤ 0.01. n = 5 mice/group.

Since aspirin at 200 mg/kg is traditionally anti-inflammatory in rodents (29), the equivalent cardioprotective dose in mice is ~14 mg/kg. After establishing a dose response, we found that chronic aspirin at levels as low as 2 mg/kg is anti-inflammatory in murine peritonitis without causing gastric lesions. Specifically, administering aspirin (2 mg/kg) to mice bearing a zymosan-induced peritonitis once daily for 10 days resulted in a significant reduction in peritoneal PMN accumulation (Fig. 6A) as well as PGE2 levels (Fig. 6B), but had little effect on proinflammatory cytokines (Fig. 6, C–E). These data support observations made in skin blisters that aspirinlow dampens innate immune-mediated responses in mouse and humans.

Discussion

We found in humans that anti-thrombotic doses of aspirin (75 mg) inhibit innate immune-mediated responses by preventing PMN and macrophage accumulation in response to tissue injury.Traditionally, the dose of aspirin used to treat inflammation in humans is 1 g, at which level it inhibits PG synthesis, including PGE2 (2, 4). It became apparent, however, that at lower doses (75 mg) aspirin is also cardioprotective through the inhibition of platelet-derived pro-thrombotic TxA2 over endothelial cell-derived PGI2 (5, 6). In this study, we advance this paradigm by showing that an additional property of cardioprotective aspirin is its ability to dampen acute inflammatory responses. We provide evidence that aspirinlow exerts its protective effects not by altering local proinflammatory cytokines or PG synthesis but in the lumen of the microvasculature by triggering 15-epi-lipoxin A4 along with increased expression of its receptor ALX. 15-Epi-lipoxin A4 then acts by inhibiting leukocyte/endothelial attachment by triggering antiadhesive NO.

Even in the absence of aspirin, there is detectable 15-epi-lipoxin A4 in the plasma and blister fluid of healthy individuals, an observation made by others using liquid chromatography-mass spectrometry-mass spectrometry (30). We conducted experiments in mice to identify the source of this constitutive 15-epi-lipoxin A4, excluding both COX1 and cytochrome P-450 with an aspirin-independent source remaining unidentified (data not included). Based on in vitro data where 15-epi-lipoxin A4 elicits NO from resting HUVECs (Fig. 5C), it is tempting to speculate that one role for endogenous 15-epi-lipoxin A4 under normal physiological conditions is to maintain vascular function by counterbalancing focal points of endothelial dysfunction by elaborating compensatory endothelial NO. Additionally, there is a correlation between temporal changes in endogenous 15-epi-lipoxin A4 in resolving blisters from volunteers not receiving aspirin, relative to PMNs from 24 to 72 h, showing that reduced PMNs are associated with elevated blister 15-epi-lipoxin A4 (r = −0.5, p = 0.005). Thus, 15-epi-lipoxin A4 is a constitutively synthesized endogenous protective lipid that may be further elevated by aspirin and which plays a role in maintaining a healthy cardiovascular system and in regulating acute inflammatory responses.

Along with inhibiting plasma TxA2, aspirinlow also inhibited plasma PGI2, effects reported previously (31). This may, at first sight, suggest contradictory effects of aspirin against platelets. However, because the production of TxA2 is key to the development of platelet aggregation to many platelet agonists and although PGI2 only acts more generally to reduce platelet reactivity, it appears that the effect of aspirin on platelet TxA2 formation outweighs its effects on vascular PGI2. In addition, platelets lack of nuclear means that while other vascular cells may regenerate COX...
after aspirin acetylation, platelets are forever inhibited. In which case, a single exposure to aspirin will inhibit platelet formation of TxA2, for its entire 2-wk existence. Although platelets facilitate PMN trafficking via GPlb/IIIa (20, 21), in this current study, we questioned the role for TxA2 in mediating this complex formation since there is no correlation between plasma/blister TxA2 levels and PMN numbers before/after aspirinlow treatment. Moreover, TxA2 can be inhibited in an animal model of acute peritonitis without impacting upon peritoneal PMN accumulation. On a separate theme, PGI2 was shown to mediate edema formation (22). However, aspirinlow had no effect on blister fluid volume. This coupled with little change in NFκB-mediated gene expression highlight clear discrepancies between studies conducted in rodents vs those in humans, possibly arising from differential dosing regimens and/or species eicosanoid receptor expression. Along these lines, the adverse immune PGEs gained over the years arose from being inhibited by NSAIDs concomitant with reduced inflammation. For instance, rather than being proinflammatory, PGEs repels leukocyte accumulation by elevating cAMP via EP2/4 receptor activation (32), while PGD2 by selective activation of the DP1 receptor dampens PMN trafficking, counterregulates proinflammatory cytokine imbalance, and triggers resolution of acute inflammation (33). Of course, PGE2 in concert with histamine and bradykinin will facilitate edema formation and mediate pain (19), but it must be highlighted that not all eicosanoids are bad, with some having no role in some models of acute inflammation in humans and others such as the resolvins and protectins being highly protective (34).

The etiology of atherosclerosis is complex, but likely starts with endothelial dysfunction, vasoconstriction, procoagulation, and leukocyte adhesion (35). This initiates an inflammatory response concomitant with migration, proliferation of local vascular smooth muscle cells, and subsequent formation of an asymptomatic alteration of the vessel wall, the “fatty streak.” The initiating stimulus is equally obuse and may involve any combination of altered responses to flow, oxidized or otherwise modified low-density lipoprotein, bacterial Ags including LPS, or proinflammatory cytokines. Nonetheless, atherosclerosis is an inflammation-driven disease. Statins lower lipids, improve endothelial function, and even slow the progression of atherosclerosis (36, 37). Indeed, in a meta-analysis of randomized trials of statins, in which aspirin was used in varying frequencies, the combination of aspirin and statins conferred greater clinical benefits than either agent alone on myocardial infarction and occlusive stroke (38, 39). However, statins are also anti-inflammatory because they trigger 15-epi-lipoxin A4 synthesis (40), endothelial NO synthase-derived NO (41), dampens proinflammatory cytokines (42) and acts as ab inhibitor of MHC class II expression by IFN-γ, thereby inhibiting T cell activation (43). Therefore, we suspect that the protective effects of antiatherogenic agents are not only in removing the putative stimulus (statins) or in maintaining vascular function (aspirin) but also in dampening the innate immune response that drives the disease. In the case of aspirinlow, we propose that in addition to inhibiting prothrombotic and vasoconstrictive TXA2, it may act by preventing PMN and monocyte adhesion to injured vessels by local production of transcellular 15-epi-lipoxin A4 biosynthesis, which in turn triggers antiadhesive NO. These effects are specific to aspirin and are not mediated by aspirin metabolites since either a single or chronic daily dosing of salicylate (200 mg/kg) did not alter PMN accumulation in a model of mouse zymosan-induced peritonitis.

Although controversial, accumulating evidence points to cardioprotective doses of aspirin and other NSAIDs being chemopreventive against colorectal cancer and possibly other cancers of the stomach, esophagus, breast, ovary, and lung (44). However, large-scale studies are necessary to assess whether aspirin can prevent cancers focusing on dose, age at which to begin treatment, and duration of therapy with randomized clinical trials being essential to definitively establish aspirin’s efficacy and safety. Moreover, it is unclear what molecular and biochemical pathways are targeted by NSAIDs in an overall attempt to identify the mechanisms by which this class of drugs may inhibit carcinogenesis. Along these lines, cancer development and growth are increasingly believed to be driven by inflammatory cells, which stimulate the growth and survival of malignant cells (45). Given that NSAIDs in general and now aspirinlow inhibit acute inflammation, it is tempting to speculate that perhaps one of the anticancer properties of aspirin may simply be the inhibition of inflammation that causes cancer.

In summary, we found that aspirinlow dampens innate immune-mediated responses in humans by triggering 15-epi-lipoxin A4 from endothelial COX2 expressed in response to local injury, which subsequently prevents leukocyte accumulation to sites of tissue injury in an NO-dependent manner (summarized in Fig. 7). We therefore highlight epi-lipoxins as novel anti-inflammatories applicable to humans and suggest that mimicking their mode of action represents a new approach to treating inflammatory diseases.
Disclosures

The authors have no financial conflict of interest.

References


