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Previews

Targeting Lipoxygenases with Care

Though fish oils possess cardio-protective, anti-inflammatory, and anti-cancer properties, their molecular and biochemical mechanism of action is lacking. In this issue of *Chemistry & Biology*, Tjonahen and colleagues [1] identify a new metabolite of eicosapentanoic acid, resolvin E2, produced by 5-lipoxygenase.

In trying to control inflammation, our traditional approach has been to target pathways thought to drive the response. However, it's becoming apparent that while such pathways are pathogenic in inflammation, paradoxically they can also be essential to bring about its resolution and maintain normal physiological functions [2]. Thus, it is the inhibition of inflammatory mediators with dual roles in health and disease that can result in side effects. The adverse cardiovascular events arising from the inhibition of inducible cyclo-oxygenase (COX-2), an enzyme that is supposedly pathogenic in inflammation, but protective in the cardiovascular system [3], provide such an example. Besides COX, it seems the lipoxygenases (LOXs) also possess an equally versatile role in pathology and physiology with their stereotypical pro-inflammatory nature increasingly overshadowed by the emerging news that LOXs mediate the anti-inflammatory and cardio-protective effects of fish oils constituents' eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). In the latest installment to this story, Tjonahen and colleagues [1] reveal a novel anti-inflammatory member of the resolvin family of EPA metabolites, namely 5-LOX-derived resolvin E2 (RvE2). In doing so, these and other authors are highlighting new anti-inflammatory, cardio-protective, and anticancerous properties of LOXs, thereby sounding a now familiar note of caution for those developing inhibitors of eicosanoid metabolism.

Before discussing the resolvins, we must recall how LOXs interact to form the lipoxins (LXs). The LX family of eicosanoids were described as transcellular metabolites of arachidonic acid (AA) generated via LOX-LOX interaction, where AA is metabolized by either 15-LOX in monocytes, eosinophils, or epithelial cells, with the intermediate eicosanoid taken up by neighboring leukocytes (PMNs) and metabolized by their 5-LOX to LXA_4 or LXB_4 [4]. LXs may also be generated by the interaction of leukocyte 5-LOX with platelet 12-LOX [5]. A third pathway may be triggered by aspirin's acetylation of COX-2, resulting not in the inhibition of COX-2, but in the generation of 15*R*-hydroxy-5,8,11,13-eicosatetraenoic acid (15*R*-HETE) from AA, which is metabolized by leukocyte 5-LOX to epimeric forms of LXA_4/B_4 (15-epi-LXA_4/B_4) [6].

Interest in fish oils and their metabolism down similar transcellular pathways as AA to the LXs was triggered, in part, by the outcome of the Greenland Eskimos study in the 1970s, which reported that fish oil rich in ω-3 EPA and DHA was associated with a reduced incidence of cardiovascular disease [7], while the GISSI prevention study reported a 45% protection against sudden cardiac death associated with taking ω-3 polyunsaturated fatty acids (PUFA) [8]. Of importance here, though each patient group in the GISSI study took daily low-dose aspirin, the contribution of aspirin plus ω-3 PUFA in the GISSI trial was not taken in to consideration. Attempts were then made to unravel whether and how fish oil-EPA and DHA could be metabolized to protective eicosanoids and if so, what their mechanism of action might be.

Using the murine air pouch model of self-resolving PMN-driven inflammation, it was found that coadministration of EPA with aspirin triggered the 5-LOX-dependent formation of 5*S*-hydroxy-eicosapentaenoic acid (5*S*-HEPE) as well as 18*R*-HEPE [9]. Similarly, inflammatory PMNs stimulated ex vivo and treated with EPA generated 18*R*-HEPE, 5*S*-HEPE along with a novel trihydroxy-containing product, called resolvin E1 (RvE1, 5*S*,12*R*,18*R*-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-eicosapentaenoic acid) [10]. Similar experiments with DHA also generated novel metabolites including 17*R*-hydroxy-DHA (17*R*-HDHA) in response to aspirin as well as 17*S*-HDHA and several related bioactive compounds without aspirin, termed the resolvin D series and protectins (for review see [11]). In addition to leukocytes, human endothelial cells expressing COX-2 or human recombinant COX-2 incubated with EPA and aspirin manufacture 18*R*-HEPE and 15*R*-HEPE, which when further cultured with activated human PMNs were converted into two classes of trihydroxy-containing EPEs, namely 5*S*,12*R*,18*R*-triHEPE (RvE1) and 5*S*,6*R*,15*R*-triHEPE (15-epi-LXA_5) [9].

Thus, as with the LXs in the context of AA metabolism, interactions between activated PMNs and vascular endothelial cells at local sites of inflammation can initiate the transcellular biosynthesis of anti-inflammatory lipid mediators from EPA and DHA. Taken together, these data show that aspirin treatment at local sites of inflammation can convert EPA via acetylated COX-2 in vascular endothelial cells to 18*R*-HEPE and 15*R*-HEPE, which is further metabolized by leukocyte 5-LOX to bioactive RvE1 and 15-epi-LXA_5. In terms of bioactivity, the LXs, resolvins, and protectins all possess what might be best described as anti-inflammatory, tissue protective, and proresolution properties, as demonstrated in experimental models of acute inflammation by controlling the phagocytosis of apoptotic leukocytes, dampening proinflammatory signals, and leukocyte trafficking [12].

In the current paper by Tjonahen and colleagues [1], these authors report the 5-LOX-dependent formation of a novel member of the resolvin E series generated during RvE1 biosynthesis that shares similar anti-inflammatory properties with RvE1. Derived from either human PMNs or human recombinant 5-LOX, the structure of this novel dihydroxyeicosanoid was found to be 5*S*, 18-dihydroxy-eicosapentaenoic acid and called RvE2, the reduction product of 5S-hydroperoxy, 18-hydroxy-EPE, an intermediate in the biosynthetic pathway of RvE1. RvE2 prevented PMN trafficking into a zymosan-induced peritonitis when given either locally or systemically suggesting

multiple sites of action and diverse pharmacological properties of this novel eicosanoid. These data further highlight the pivotal role of 5-LOX in the generation of anti-inflammatory lipid mediators.

Some products of LOX metabolism, the leukotrienes, are undeniably proinflammatory while others are protective. For instance, 5-LOX-generated leukotriene B_4 is one of the most potent chemoattractants for PMNs [13], a property that made it a target for drug development either in the form of selective inhibition or as a cotarget along with COX, so-called dual COX/LOX inhibitors. However, 5-LOX-derived LXs and resolvins are emerging as some of the most important endogenous regulators of the innate immune systems where, in addition to exerting proresolution effects, they are critical in orchestrating inflammatory responses to parasite infection [14]. 5-LOX also enhances PMN phagocytic clearance of bacteria from the lung [15], alveolar microbicidal capacity [16], and resistance against *Klebsiella*-induced pneumonia [17]. In this setting of infection and immunity, 5-LOX is essential to our immune defense system. Thus, in the same way that COX-2 plays multiple roles in inflammation and normal physiology, 5-LOX can be proinflammatory or protective depending on the disease. It is these findings that remind us that when developing inhibitors of AA metabolism, we must be highly cognizant of the diverse and often opposing roles eicosanoids play in host defense and that such inhibitors must be tailored for diseases in which that pathway plays a pathogenic role. This applies not only to the inflammatory response to infection and/or injury, but also to the recently identified role LOXs play in carcinogenesis [18].

Advances in unveiling the molecular mechanisms behind the benefits of aspirin and fish oils have underscored the critical importance that the enzymes (COX and LOX) central to lipid mediators metabolism (AA, EPA, and DHA) have in health and disease. Products of these pathways were once maligned and the target of drug discovery spawning the era of NSAID drug development. While NSAIDs have provided us with great benefit in terms of anti-inflammation and pain relief, they were not without their side effects showing that some LOX and COX products are protective. The LXs and the recently discovered resolvins and protectins have improved the reputation eicosanoids have gained over the years in the field of inflammation research, and pointed out the benefits of some COX or LOX products. Thus, developing inhibitors for COX/LOX enzymes will not be efficacious for all inflammation-driven diseases and may even be detrimental to some. The point is that eicosanoids are pathogenic in some diseases, protective in others while at the same time being essential for normal physiological processes. It is this complexity of function that must be borne in mind when developing inhibitors of eicosanoid metabolism in order to minimize side effects.

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Selected Reading

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