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The expansive role of oxylipins on platelet biology

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Abstract

In mammals, three major oxygenases, cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450 (CYP450), generate an assortment of unique lipid mediators (oxylipins) from polyunsaturated fatty acids (PUFAs) which exhibit pro- or anti-thrombotic activity. Over the years, novel oxylipins generated from the interplay of the oxygenase activity in various cells, such as the specialized pro-resolving mediators (SPMs), have been identified and investigated in inflammatory disease models. Although platelets have been implicated in inflammation, the role and mechanism of these SPMs produced from immune cells on platelet function are still unclear. This review highlights the burgeoning classes of oxylipins that have been found to regulate platelet function; however, their mechanism of action still remains to be elucidated.

Keywords

Lipoxygenase; Cyclooxygenase; Oxygenases; Eicosanoids; Prostaglandins; Thrombosis

Introduction

Cardiovascular disease remains the leading cause of mortality globally accounting for nearly 1 in 3 deaths annually [1]. Platelet activation leading to clot formation and thrombosis is an essential component of both the hemostatic and thrombotic responses in the blood following physiological and pathophysiological disturbance of the endothelium lining the vessel wall [2]. The inability to properly regulate platelet reactivity often leads to atherothrombotic events, including myocardial infarction and stroke. Recent work in the field has uncovered a number of lipid products, eicosanoids, derived from ω -3 or ω -6 polyunsaturated fatty acids (PUFAs) that significantly regulate and alter platelet function. The PUFAs include arachidonic acid (AA), linoleic acid (LA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), and dihomogamma-linolenic acid (DGLA). Understanding how these newly identified lipids fit into the overall regulation of platelets in the vessel will aid in our understanding of lipidplatelet interactions, often resulting from altered diet or fatty acid supplementation, that play key roles in the ability of the platelet to form a hemostatic “plug” following vascular injury or alternatively form an

occlusive thrombus following patho-physiologic insult to the vessel. Finally, understanding how these lipids and lipid products are generated and regulate platelet reactivity should reveal novel targets for therapeutic intervention to prevent thrombosis while limiting the risk for bleeding following vessel injury. Thus, this review will be limited to describing the various lipids and bioactive lipid products shown to regulate platelet function and modulate hemostasis and thrombosis in the vessel.

PUFAs are generally inert and depend on oxygenase activity to generate a wide array of structurally distinct bioactive fatty acids metabolites. The formation of lipid products is typically initiated by stimulation of the cell that results in an increase in intracellular calcium. This calcium flux results in translocation of cytosolic phospholipase A2 (cPLA₂) to the lipid membrane where it cleaves the fatty acid from the sn-2 position of the phospholipids to release free fatty acids for oxidation in the cell. Once cleaved from the lipid membrane, the freed fatty acids can be metabolized by cyclooxygenase (COX), lipoxygenase (LOX) or cytochrome P450 (CYP450) to form oxidized lipids (oxylipins). Oxylipins have been thought to predominantly function by regulating cellular properties and signaling through one of three pathways. The first involves binding to G protein-coupled receptors (GPCRs) to further propagate intercellular signaling. Secondly, fatty acids or their oxylipins can directly interact with peroxisome proliferator-activated receptors (PPARs) within the cell. While fatty acids are thought to be weak activators of PPARs, when they accumulate in the vicinity of the PPAR, reports have shown their affinity for activating PPAR signaling is significantly increased [3]. The third regulatory mechanism utilized by fatty acids and oxylipins in the platelet is direct inhibition of oxylipin-producing enzymatic pathways or further metabolic transformation of lipids within the cell (Fig. 1). The review will cover the oxygenase pathways, classes of structurally distinct oxylipins, and their biological effects on the platelets.

Cyclooxygenase

Cyclooxygenase (COX) exists in two isoforms, COX-1 and COX-2 in the body; however, the platelet expresses primarily COX-1, and its inhibition is thought to be a primary target for reduction of platelet reactivity in the patients with cardiovascular risk. COX activation primarily results in the generation of prostanoids (prostaglandins (PGs) and thromboxanes (TXs)) derived from PUFAs that are responsible for maintaining either physiological or pathophysiological states, such as inflammation and tumorigenesis [4]. This section describes the select prostanoid lipids generated from the PUFAs through the COX pathway that regulate platelet function.

COX-derived metabolites and their regulatory roles on platelet function

COX transforms AA to series 2 PGs (PGE₂, PGD₂, PGI₂) and thromboxanes (TX) A₂ that can exhibit either pro-thrombotic or anti-thrombotic modulation of platelet function (Table 1) [5, 6]. In terms of thrombosis, TXA₂, when formed, is released and acts through the thromboxan receptor (TPα), which is coupled to Gα_q and Gα₁₃ and functions to amplify platelet activation leading to enhanced aggregation and thrombosis [7]. In contrast, PGD₂ derived mainly from mast cells, leukocytes, and some platelets [8], had been shown to

dampen platelet activation [9–11] through its binding to the DP1 receptor and subsequent elevation of cAMP [12–14]; however, there are evidence that PGD₂ can also directly activate PPARs [15]. PGD₂ can be further dehydrated to PGJ₂, ¹²-PGJ₂, and 15-deoxy- ^{12,14}-PGJ₂, and inhibit platelet activation through a number of signaling pathways including activation of PPARs [16, 17]. While PGD₂ derivatives are known PPAR ligands, the role of PPARs in platelet activation has not been fully elucidated. Similar to PGD₂, PGI₂ (prostacyclin), a well-characterized vasodilator [18], has been shown to activate adenylate cyclase in the platelet via the prostacyclin (IP) receptor and in turn antagonizes platelet aggregation at sites of injury [6, 19]. Although PGI₂ has been shown to exert antiplatelet effects in vivo and is also clinically available to treat cardiovascular related diseases, a major concern of the use is the increased occurrence of hypotension. Moreover, PGE₂ exhibits pleiotropic effects by which it can induce both proand anti-platelet responses depending on the concentration [20, 21] through the binding of one of more of its prostaglandin receptors: EP1, EP2, EP3 and EP4 [22].

DGLA is an ω-6 PUFA that can be acquired through supplementation of γ-linolenic acid (GLA) in the diet. COX converts DGLA to series 1 prostaglandins (PGD₁, PGE₁) and TXA₁ [23, 24], which inhibit platelet function in vitro and in vivo [25]. These DGLA-derived COX prostanoids exerted their anti-platelet action by activating the Gα_s-coupled GPCRs, prostaglandin (EP₂ and EP₄) or IP receptors.

Similar to DGLA, COX acts on EPA, an ω-3 PUFA, to generate anti-inflammatory [26] lipid mediators, series 3 PGs and TXs [27, 28]. EPA-derived metabolites of COX (PGE₃, PGD₃, PGI₃) inhibit platelet aggregation [29–32] and P-selectin expression induced by platelet activating factor (PAF) as well as inhibiting platelet-rich plasma (PRP) [7]. Evidence for the series 3 PGs receptors is scant. PGE₃ had been suggested to be a partial agonist of the EP receptors in human kidney cells with varying degree of affinity potencies [29], inducing secondary messenger actions. For instance, PGE₃ mediates Gα_q activation and subsequent intracellular calcium release through the EP₁ receptor. While Gα_s activation and enhanced cAMP formation were observed in EP₂ and EP₄ overexpressed cells treated with PGE₃, EP₃ ligand binding resulted in reduced cAMP generation and augmented inositol triphosphate (IP₃) formation coupled to Gα_i activation [29]. Platelets treated with EP₃, EP₄, IP and receptor antagonists (DG-41, ONO-AE3-208, and CAY10441, respectively) demonstrated that PGE₃ acted as an antagonist to the EP₃ to further inhibit platelet function, but with no effect mediated by the IP receptor agonist on platelet reactivity. In contrast, CAY10441 reversed the ability of PGE₃ to inhibit platelet function [30]. These studies suggest that PGE₃ is also capable of producing multiple and simultaneous effects, resulting in either pro- or anti-thrombotic outcome.

Both PGD₃ and PGI₃ are deemed to behave similarly to PGD₂ and PGI₂ agonists as well as exerting through the same cognate receptors to increase cAMP. PGD₃ is also shown to be further dehydrated PGJ₃, ¹²-PGJ₃, and 15-deoxy- ^{12,14}-PGJ₃ [33] and act on DP receptor or possibly function through the PPARs [34]. PGI₃ is an unstable analog of PGI₂ that exerts its action on the IP receptor, but has been observed to activate PPAR [35]. TXA₃ is presumed to act on EP₂ and EP₄ receptors to enhance cAMP [31] to inhibit platelet function

similar to TXA₁, but additional cell line and pharmacological models are required to verify this assumption.

Lipoxygenases

Mammalian lipoxygenases (LOXs) constitute the following heterogeneous group of lipid-peroxidizing enzymes that are categorized accordingly to their positional specificity of AA oxygenation: 5-LOX, 12-LOX, and 15-LOX. While these enzymes are expressed in a number of cells, they each produce oxylipins that function in part to regulate platelet activity, hemostasis, and thrombosis.

5-Lipoxygenase (5-LOX)

5-LOX is best known for its ability to produce leukotrienes (LTs) [28]. LTs are synthesized in myeloid cells (eosinophils, neutrophils, mast cells, macrophages, monocytes, dendritic cells, basophils, and B-lymphocytes) that are involved in inflammatory, immune, and allergy responses. 5-LOX also produces non-LT products (5-hydroxyeicosatetraenoic acid (5-HETE)) and two structural forms of LTs, which consist of cysteinyl-free (LTA, LTB) and cysteinyl-LTs (LTC, LTD, LTE, LTF) [36] (Fig. 2, Table 1). The type of PUFA substrate being oxidized dictates the class of LTs formed to modulate inflammatory response and vascular tone. While platelets lack 5-LOX, there are numerous studies suggesting the interplay between leukocytes and platelets through their eicosanoid production [37] by which platelets can modulate immunological response or vice versa [38, 39].

5-LOX-derived metabolites and their regulation of platelet function

Cellular induction by various stimuli (chemotactic agents, immune complexes, bacterial peptides) leads to translocation of 5-LOX to the membrane [40–42] to convert AA to 5-HETE. 5-HETE can further be converted by 5-hydroxyeicosanoid dehydrogenase to form 5-oxo-6,8,11,14-eicatetraenoic acid (5-oxo-EETE) (Fig. 2), or dehydrated to a member of series 4 epoxide intermediate leukotriene, LTA₄ [43].

Previous studies have demonstrated a platelet role in the transcellular metabolism of LTs released by neutrophils, enhancing platelet-neutrophil interactions. The interplay between platelet-adherent leukocyte interactions has been implicated in chronic inflammatory diseases in patients, including aspirin-exacerbated respiratory disease (AERD). While neutrophils lack LTC₄ synthase to convert LTs, platelets have been shown to possess abundant LTC₄ synthase [44–47] to convert unmetabolized LTA₄ secreted from neutrophils or monocytes to LTC₄ requiring P-selectin-dependent interaction [48–50]. Through this interaction, transcellular metabolism of LTC₄ is increased, resulting in exacerbated inflammatory response [51–53].

Although the secreted LTs function principally to activate and recruit additional neutrophils to propagate inflammatory responses in allergy or asthma, platelets have also been shown to respond to LTs. Human PRP pretreated with exogenous LTs (LTE₄, LTD₄, LTC₄) for example, showed potentiation in aggregation and TXB₂ production following sub-threshold stimulation with thrombin and epinephrine [54]. The effects of LTs potentiation of human

PRP aggregation and TXB₂ generation are presumed to be mediated through the platelet cys-LT receptors, type 1 and type 2 (CysLT₁R and CysLT₂R) [55], which are involved in chemokine, RANTES, release in inflammation [55–59]. The functional importance of CysLT₂R in platelet function was demonstrated in transgenic mice deficient in CysLT₂R (*Cysltr2*^{−/−}) indicating CysLT₂R expression was required for low nanomolar LTC₄ induction of P-selectin, ADP, and TXB₂ release from platelets [60]. On the other hand, LTE₄ and LTD₄ did not augment P-selectin expression in wild-type, *Cysltr1*^{−/−} or *Cysltr2*^{−/−} platelets, suggesting metabolite specificity for these biochemical regulatory steps. Interestingly, LTC₄ induction of P-selectin expression was also observed to be markedly impaired in purinergic receptor P2Y₁₂ knockout mouse platelets. Together, these studies suggest that P2Y₁₂-targeted thienopyridine drugs used for the management of cardiovascular ischemic events may also interfere with the LTC₄/CysLT₂R-dependent pathway of platelet activation.

The biological activity of 5-HETE on platelet function has been controversial. In vitro studies showed 5-HETE inhibits the binding of the radiolabeled thromboxane mimetic, [¹²⁵I] BOP, to the PGH₂/TXA₂ receptor in washed human platelets with IC₅₀ values greater than 25 μM. This observation suggests that 5-HETE directly inhibits platelet activation through direct competition with PGH₂/TXA₂ [61]. Conversely, thrombin-induced platelet aggregation and ADP release was shown to be potentiated with 30 μM of 5-HETE [62]. In light of clinical studies being conducted with the use of 5-HETE inhibitors, it will be of high importance to delineate the potential effect of these inhibitory strategies targeting 5-HETE production on platelet reactivity and hemostasis.

12-Lipoxygenase (12-LOX)

Both 12*S*-LOX and 12*R*-LOX isoenzymes, which generate distinct chiral metabolites from PUFAs, are expressed in selective mammalian tissues and cells. 12-LOX is further classified as platelet, leukocyte, or epithelial-type. Platelet-type 12-LOX is expressed in all mammalian species, whereas the leukocyte-type 12-LOX is found in murine, porcine, and bovine, but not in humans or rabbits [63–65]. Conventionally, 12-LOX is characterized for its ability to convert AA to 12-hydroperoxyeicosatetraenoic acid (12-HpETE), which is rapidly reduced to 12-hydroxyeicosatetraenoic acid (12-HETE) (Fig. 2). To date, majority of the platelet related studies have focused on 12*S*-LOX products, since no 12*R*-LOX products have been found to regulate platelet function. Thus, for the purpose of this review, only the *S* configuration metabolites of 12-LOX will be discussed.

Regulation of platelet function by 12-LOX-derived metabolites

The major metabolite of 12-LOX, 12-HETE, has been described to have both anti-thrombotic and prothrombotic effects. The anti-thrombotic effect of 12-HETE was first implicated by its direct inhibition of neutrophil PLA₂ activity by which the availability of AA was reduced in vitro [66]. In support, exogenous 12-HETE suppressed collagen-induced liberation of AA in bovine platelets [67]. Platelets from 12-LOX-deficient mice were hyper-responsive to aggregation induced by ADP, and this phenomenon was reversed by 12-HETE treatment [68]. 12-HETE and 12-HpETE were also reported to inhibit PGH₂- and collagen-

induced platelet aggregation [69–71] as well as prevent binding of PGH₂ and TXA₂ to their cognate receptors [68].

In stark contrast, 12-HpETE and 12-HETE have been demonstrated to potentiate platelet activation and aggregation. Exogenous 12-HpETE, at nanomolar concentrations, stimulated platelet p38 mitogen-activated protein kinase, as well as phosphorylation of cytosolic PLA₂, increased TXB₂ and dense granule secretion [71–74]. 12-HETE was also shown to potentiate bovine platelet aggregation induced by thrombin as well as inhibiting PGE₁-induced elevation of cAMP. Pharmacological inhibition and genetic ablation of 12-LOX have also demonstrated the importance of 12-HETE in potentiation of platelet activation [74–76].

The pro-thrombotic effect of 12-HETE is thought to be mediated through its esterification into the lipid membrane following formation in the platelet, which results in enhanced tissue factor-dependent thrombin generation in the vessel [77]. It is also possible that 12-HETE could be mediating its effect on platelet function through high affinity binding to an orphan GPCR, GPR31 (12-HETER) [78], which was originally discovered in cancer cells to promote survival and metastasis as well as neuronal cells that modulate voltage-sensitive calcium channels [79]. Alternatively, 12-HETE had also been shown to enhance and activate peroxisome proliferator-activated receptor γ (PPAR γ), a member of the nuclear hormone receptor family of ligand-dependent transcription factors [80]. To date, the expression of 12-HETER in platelets has not been confirmed.

While the predominant metabolite, 12-HETE, has been shown to have contradicting roles, other 12-LOX derived metabolites from EPA, DHA, DPA, and DGLA (12-HEPE, 11/14-hydroxydocosahexaenoic acid (11/14-HDHA), 11/14-hydroxydocosapentaenoic acid (11/14-HDPA), and 12-hydroxyeicosatrienoic acid (12-HETrE)) [70, 76, 81] have been shown to exert anti-platelet or anti-thrombotic in vivo effects. These metabolites vary in potency and ability to be synthesized. For instance, only trace amounts of 11/14-HDHA (Fig. 3) were detected in EPA or DHA pretreatment compared to 12-HEPE and 12-HETE following thrombin stimulation in platelets, suggesting that higher concentrations of DHA are needed for platelet inhibition [82]. Additionally, DPA is observed to exert its anti-platelet effect [83] through inhibition of COX-1 activity by 11/14-HDPA [84] (Fig. 4).

Though previous studies have implicated the 12-LOX-derived metabolites in cardioprotection through the dampening of platelet activation, there were no direct in vivo evidence to support those claims. More recently, the role of platelet 12-HETrE on thrombosis and underlying mechanisms were investigated in vivo and ex vivo. Mice intravenously administered with 6 mg/kg of 12-HETrE or 50 mg/kg DGLA were protected from thrombus accumulation at the site of arteriole vessel injury [85]. The anti-platelet effects of DGLA in vivo were also shown to be dependent on the presence of functional platelet 12-LOX in mouse platelets. For instance, even though mice lacking 12-LOX (*ALOX12*^{-/-}) had attenuated thrombus formation within the vessel following laser injury, DGLA treatment did not further prevent thrombus growth in the *ALOX12*^{-/-} mice compared to the wild-type. This demonstrated that 12-LOX was required for DGLA-mediated inhibition of platelet activation and thrombus formation. Finally, the anti-platelet effect of

12-HETrE was shown to be mediated through a $G\alpha_s$ -linked GPCR, which activates adenylyl cyclase and subsequent downstream effectors to inhibit platelet activation.

15-Lipoxygenase (15-LOX)

Two forms of 15-LOX isoforms exist in mammalian tissues, leukocyte-type 15S-LOX (15-LOX-1) and epidermis-type 15-LOX type B (15-LOX-2) [86–88]. Tissue distribution of 15-LOX-2 is limited when compared to that of 15-LOX-1. 15-LOX-1 is expressed in eosinophils, leukocytes, reticulocytes, macrophages, dendritic, epithelial cells (bronchial, corneal, and mammary) [89, 90], whereas, 15-LOX-2 is predominantly found in the skin, prostate, lung, and cornea [91].

15-LOX-derived metabolites and regulation of platelet function

While the existence of 15-LOX-1 and –2 in platelets is questionable, platelets have demonstrated the ability to generate the 15-LOX oxylipin products, 15-hydroxyeicosatetraenoic acid (15-HETE), 8,15-dihydroxyeicosatetraenoic acid (8,15-diHETE), and 14,15-dihydroxyeicosatetraenoic acid (14,15-diHETE) from AA (Fig. 2) [87], and 17-hydroxydocosahexaenoic acid (17-HDHA) [92] from DHA (Fig. 3, Table 1). Both 15-HETE and 8,15-diHETE were shown to inhibit platelet aggregation induced by collagen, ADP, epinephrine, AA, or prostaglandin H2 analog [93–95]. Conversely, 15-HETE and 15-HpETE were also demonstrated to enhance whole blood aggregation and thrombin generation in the presence of macrophages [96]. 15-HETE (between 1 and 100 nM) enhanced thrombin-stimulated platelet aggregation, ADP release, and secondary messengers (IP₃, diacylglycerol, and intracellular calcium) production [62]. Similarly, 17-HDHA was shown to potentiate ADP-induced platelet aggregation and spreading, but inhibited α -granule secretion [97]. The latter data suggests that 15-LOX products can function as procoagulant mediators.

Both 13-hydroxyoctadecadienoic acid (13-HODE) and 15-hydroxyeicosatrienoic acid (15-HETrE), major metabolites of 15-LOX derived from linolenic acid (LA) (Fig. 4) and DGLA (Fig. 3), respectively, were shown to inhibit rabbit [98] and human platelet aggregation [99]. Additionally, 13-HODE was demonstrated to inhibit thrombin-induced TXB₂ and 12-HETE production in platelets as well as platelet adherence to endothelial cells in vitro [99, 100]. Interestingly, 15-HETrE exhibited biphasic effects on platelet aggregation in which low concentrations potentiated and higher concentrations inhibited platelet aggregation [101].

Cytochrome P450

Cytochrome P450 enzymes (CYP450s) belong to a large group of oxygenases, with at least 57 putatively functional subfamilies in humans and upwards of 102 in mice [102, 103]. CYP450s are expressed primarily in the liver, with some detection in the heart, lung, vasculature, kidney, and gastrointestinal tract. Traditionally, these membrane-bound and hemecontaining oxygenases are recognized for their xenobiotic metabolism and detoxification of drugs; however, multifaceted functions have also been uncovered. These enzymes are also involved in the metabolism of eicosanoids from fatty acids, vitamin D3

synthesis, biosynthesis of cholesterol and bile acids, and synthesis and metabolism of steroids [104].

Regulation of platelet function by CYP450 epoxygenase and hydroxylase-derived metabolites

AA can be synthesized by endothelial CYP450 epoxygenase into a number of epoxyeicosatrienoic acids (5,6-epoxyeicosatrienoic acid (5,6-EET), 8,9-epoxyeicosatrienoic acid (8,9-EET), 11,12-epoxyeicosatrienoic acid (11,12-EET), and 14,15-epoxyeicosatrienoic acid (14,15-EET)), which are further catalyzed to dihydroxyeicosatrienoic acids (5,6-diHETrE, 8,9-diHETrE, 11,12-diHETrE, 14,15-diHETrE) by soluble epoxide hydrolase (sEH). Endothelial CYP450 ω -hydroxylase also acts on AA to generate 19-hydroxyeicosatetraenoic acid (19-HETE) and 20-hydroxyeicosatetraenoic acid (20-HETE) to maintain vascular tone and hemostasis (Fig. 2). Early studies demonstrated that many epoxygenase isomers ranging from 1 to 10 μ M, regardless of their regiochemical, geometric, and stereochemical structures, were effective at inhibiting human platelet [105–107] or PRP [108] aggregation independent of TXB₂ and cAMP formation [107]. However, more recent reports suggest that 11,12-EET (ranging from 1 to 10 μ M) do not inhibit platelet aggregation stimulated with collagen, ADP or a thrombin-receptor activating peptide [109]. The conflicting observations from several research groups will require further study to determine if this class of EETs is pro-thrombotic, anti-thrombotic, or is bi-functional depending on platelet conditions (PRP or isolated platelets) and agonist stimulation used.

EETs (5,6-EET, 11,12-EET, 8,9-EET, and 14,15-EET) have additionally been demonstrated to hyperpolarize platelets through the activation of calcium potassium channels resulting in decreased ADP-induced P-selectin expression on platelet surface as well as platelet adhesion to cultured endothelial cells under physiological shear stress [110]. To further support CYP450-derived products from endothelial cells regulate platelet function, supernatant releasate from bradykinin-stimulated cultured endothelial cells overexpressing CYP2C9 were shown to inhibit platelet adhesion. Finally, in vivo anti-thrombotic effects of CYP2C9-derived metabolites were demonstrated in the arteriolar wall of hamster. Hamsters administered with CYP2C9 inhibitor, sulfaphenazole, at doses known to block endothelium-derived hyperpolarizing factor-dependent dilations, significantly enhanced platelet-vessel wall interactions. The firm adhesion of platelets to vessel wall was reversed when superfused with 10 μ M of 11,12-EET [111].

The CYP450 ω -hydroxylase product of AA, 19-HETE, was found to be an orthosteric prostacyclin receptor agonist that inhibited mouse platelet aggregation. To verify that 19(*S*)-HETE, and not its regioisomer 19(*R*)-HETE, was responsible for binding to the prostacyclin receptor and inhibiting platelet activity, a megakaryocyte cell line, MEG-01 [112], with intact G α_s expression was shown to enhance cAMP formation following dose-dependent 19(*S*)-HETE treatment. Additionally, COX-1/2 inhibition of COS-1 human IP receptor expressing cells did not interfere with the ability of 19(*S*)-HETE to directly induce cAMP formation in MEG-01. Blocking the IP receptor with the selective prostacyclin receptor inhibitor, Cay104401, prevented 19(*S*)-HETE stimulation of cAMP generation in MEG-01

cells. Similarly, 19(*S*)-HETE was able to displace ³H-iloprost in COS-1 cells expressing IP receptor, demonstrating that 19(*S*)-HETE behaved as a competitive agonist binding to the same domain of the IP receptor as iloprost (and likely PGI₂). These observations were confirmed when pretreatment of IP deficient mouse (*Ptgir*^{-/-})_platelets with 3 μM 19(*S*)-HETE failed to block thrombin-induced platelet aggregation.

Another eicosanoid found to have potent inhibitory properties against the platelet is 20-HETE. This eicosanoid was found to have a potent effect on inhibiting human platelet aggregation and TXB₂ formation induced by AA, calcium ionophore, A23187, and the TXB₂ mimetic without affecting thrombin-induced aggregation [113]. The proposed inhibitory effect of 20-HETE on platelet activation was also presumed to be its antagonism of the PGH₂/TxA₂ receptors [113, 114]. Aside from receptors antagonism, 20-HETE was also shown to be further metabolized by COX-1 and 12-LOX to the inactive 11,12-dihydroxyeicosatetraenoic acid (11,12-diHETE) and 12,20-dihydroxyeicosatetraenoic acid (12,20-diHETE), respectively, in human platelets [113]. Thus, it is possible that at least some of the observed anti-platelet effect could be attributed to its metabolic transformation to the diHETEs.

Upon dietary supplementation of ω-3 or -6 PUFAs, AA-derived products of CYP450 epoxygenase are partially replaced by EPA and DHA-derived epoxyeicosatetraenoic acids (EEQs) and (epoxydocosapentaenoic acids (EDPs), respectively [115]. In the case of CYP450 epoxygenase-derived metabolites of EPA (8,9-EEQ, 11,12-EEQ, 14,15-EEQ, 17,18-EEQ) (Fig. 4) and DHA (7,8-EDP, 10,11-EDP, 13,14-EDP, 16,17-EDP, and 19,20-EDP) (Fig. 3), all metabolites were shown to inhibit AA-induced platelet aggregation [116] (Table 1). Even though all the diols produced by sEH conversion of the EEQs (8,9-DiHETE, 11,12-DiHETE, 14,15-DiHETE, 17,18-DiHETE) and EDPs (7,8-DiHDPA, 10,11-DiHDPA, 13,14-DiHDPA, 16,17-DiHDPA, 19,20-DiHDPA) inhibited platelet aggregation; they were less potent at inhibiting platelet aggregation than the parent epoxides.

Although several of these studies have demonstrated EET and hydroxylate metabolites are derived from cells with intact CYP450, preformed epoxides and 20-HETE of AA have been found as integral components of human platelet membrane [106]. Thus, it is possible that circulating EETs and their diol products, DHETs, and hydroxylates are avidly taken up by platelets and endothelial cells [117, 118]. These products can be released during receptormediated hydrolysis of platelet phospholipids [106] or further metabolized by COX [119] and LOX [113]. For instance, once stimulated, EETs are de-esterified in platelets and released to influence the migration pattern of nearby neutrophils [106, 120]. In contrast, CYP450 ω-hydroxylase inhibitor, HET0016, blocked angiotensin and endothelin-stimulation of 20-HETE secretion from platelets, suggesting that CYP450 isoforms exist in the platelet [115]. Based on study discrepancy, the endogenous expression of CYP450 in the platelet has not been confirmed and will need to be definitively determined before platelet generation of epoxide or hydroxylate products can be assigned to the platelet itself or alternatively if these products are presented to the platelet from other blood cells, including the endothelium and neutrophils.

The interplay of oxygenases and generation of specialized pro-resolving lipid mediators (SPMs)

Over the past decade, studies have focused on the role of specialized pro-resolving lipid mediators (SPMs) on preventing excessive inflammation, infection, and wound repair, through their ability to attenuate or dissipate chemotactic and pro-inflammatory signals. SPMs, which include lipoxins (LX), D and E series resolvins (Rv), (neuro) protectins (PD), and maresins (MaR), are synthesized by the sequential action of LOXs on PUFAs to resolve and restrain inflammation [100] (Fig. 5). Despite the prevalence of platelet involvement in the inflammatory process, little is known on how and whether SPMs play a direct role on regulation of platelet function.

Lipoxins A (LXA₄) and B (LXB₄) were one of the first SPMs to be identified from the combination of 5- and 15-LOX in human leukocytes [43] as well as neutrophil-derived 5-LOX and platelet 12-LOX [121–124] from AA. Although platelets express LXA₄ receptor (ALX) [125], LXA₄ does not directly inhibit platelet aggregation induced by ADP [98] and bacterial infection [98]. Alternatively, aspirin-triggered lipoxin (ATL), 15(*R*)-epi-lipoxin A₄ (15(*R*)-epi-LXA₄) [126], is indirectly derived from the acetylated COX-2 metabolism of AA. Both LXA₄ and 15(*R*)-epi-LXA₄ had been demonstrated to modulate neutrophil-platelet aggregation through ALX; however, it remains unclear whether these lipoxins can directly regulate platelet function based on limited studies.

Resolvin E₁ (RvE₁), synthesized by acetylated COX-2 or sequential CYP450 and 5-LOX activity of EPA, was demonstrated to inhibit human PRP aggregation stimulated by ADP and TXB₂, but not collagen [127]. RvE₁ was also shown to inhibit P-selectin expression on activated platelets and platelet actin polymerization, without affecting calcium mobilization. The observed anti-platelet effects of RvE₁ were shown to act through the ChemR23 receptor on the surface of platelets [128]. In contrast, resolvin D₁ (RvD₁) and its intermediary precursor, 17-HDHA, derived from 15-LOX and 5-LOX synthesis of DHA, potentiated ADP-mediated platelet aggregation and spreading on fibrinogen. ADP-mediated release of α-granules in platelets were not affected by RvD₁ and 17-HDHA; however, thrombin stimulation of α-granules was significantly attenuated by these SPMs [129]. RvD₁ is presumed to exert its effect on its cognate receptor, GPR32, on the platelet surface. Thus far, while data support a role for platelets in the generation of inflammatory markers, the mechanism by which resolvins regulate platelets remains unclear.

Maresin 1 (MaR1) is derived from the biosynthesis of DHA by both neutrophil 15-LOX and platelet 12-LOX [98]. Early studies demonstrated MaR1 anti-inflammatory and proresolving properties in lung catabasis. While MaR1 was shown to potentiate platelet aggregation and spreading, it also dampened pro-inflammatory and pro-thrombotic granules, suggesting MaR1 differentially regulates platelet function through a mechanism that has not been fully elucidated to date.

Protectin DX (PDX) belongs to a group of di-oxygenated derivatives of PUFAs, called poxytrins [130]. PDX is an isomer of neuroprotectin D1 (PD1) [131], which was originally discovered to attenuate brain ischemia-reperfusion [132, 133]. The PDX isomer was

demonstrated to be biologically less potent than PD1 in the resolution of inflammation; however, effective in inhibiting collagen-, AA-, and thromboxane-induced platelet aggregation through inhibition of COX-1, at nanomolar concentrations [134].

Discussion and future implications

Regulation of platelet function is a key step in both physiological and pathological hemostatic processes. While inhibition of platelet activation remains a first-line approach for prevention of myocardial infarction and stroke, morbidity and mortality due to cardiovascular diseases and stroke remain the top causes of death globally. Hence, a greater understanding of the regulators of platelet function in vivo will significantly aid in the development of novel treatments to prevent unwanted clotting and occlusive thrombosis. Lipids and their oxylipins have long been known to regulate platelet function; however, until recently, the breadth of regulators and functions they control in the platelet have not been fully appreciated.

In this review, we have highlighted some of the major breakthroughs in identifying oxylipins we now know have direct effects on the platelet (Fig. 6). The use of genetic manipulation and pharmacological tools in both mouse and cellular models to determine the oxygenases and their lipid contributions to vascular and platelet functions has progressed considerably over the last two decades. These tools have greatly enhanced our understanding of the varying roles of oxylipins in platelet biology; however, these studies are still limited to another layer of complexity. The difficulty in targeting the precise pathway or oxylipins associated with pathophysiological disease states stems from the source of oxylipins generated by the involvement of multiple oxygenase enzymes localized in different organs and cell types. Even with the use of pharmacological tools to determine the contributions for each oxygenase metabolites to platelet function, selectivity of drug target still remains a major setback. For instance, chemical compounds designed to inhibit CYP epoxygenase enzymes also antagonized CYP hydroxylase enzymes activity. Therefore, interpretation of results with pharmacological inhibitors should be taken with caution. Future work will thus focus on further delineating the full breadth and diversity of oxylipins regulating platelet function ex vivo and in vivo and determining the mechanism(s) by which they exert their regulatory function on the human platelet to develop newer pharmacological approaches for targeting pathways involved in the regulation of platelet to address pathological conditions whereby normal regulation of hemostasis and thrombosis has become dysfunctional.

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References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. *Circulation*. 2015; 131:e29–322. [PubMed: 25520374]
2. Jackson SP. Arterial thrombosis—insidious, unpredictable and deadly. *Nat Med*. 2011; 17:1423–1436. [PubMed: 22064432]

3. Tourdot BE, Ahmed I, Holinstat M. The emerging role of oxylipins in thrombosis and diabetes. *Front Pharmacol*. 2014; 4:176. [PubMed: 24432004]
4. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. *J Lipid Res*. 2009; 50(Suppl):S29–S34. [PubMed: 18952571]
5. Chandrasekharan JA, Marginean A, Sharma-Walia N. An insight into the role of arachidonic acid derived lipid mediators in virus associated pathogenesis and malignancies. *Prostaglandins Other Lipid Mediat*. 2016; 126:46–54. [PubMed: 27450483]
6. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol*. 2011; 31:986–1000. [PubMed: 21508345]
7. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutr Metab*. 2012; 2012:539426. [PubMed: 22570770]
8. Nakahata N. Thromboxane A2: physiology/pathophysiology, cellular signal transduction and pharmacology. *Pharmacol Ther*. 2008; 118:18–35. [PubMed: 18374420]
9. Oelz O, Oelz R, Knapp HR, Sweetman BJ, Oates JA. Biosynthesis of prostaglandin D2. 1. Formation of prostaglandin D2 by human platelets. *Prostaglandins*. 1977; 13:225–234. [PubMed: 847230]
10. Whittle BJ, Moncada S, Vane JR. Comparison of the effects of prostacyclin (PGI₂), prostaglandin E1 and D2 on platelet aggregation in different species. *Prostaglandins*. 1978; 16:373–388. [PubMed: 364545]
11. Song WL, Stubbe J, Ricciotti E, Alamuddin N, Ibrahim S, Crichton I, Prempeh M, Lawson JA, Wilensky RL, Rasmussen LM, et al. Niacin and biosynthesis of PGD(2) by platelet COX-1 in mice and humans. *J Clin Invest*. 2012; 122:1459–1468. [PubMed: 22406532]
12. Bushfield M, McNicol A, MacIntyre DE. Inhibition of platelet-activating-factor-induced human platelet activation by prostaglandin D2. Differential sensitivity of platelet transduction processes and functional responses to inhibition by cyclic AMP. *Biochem J*. 1985; 232:267–271. [PubMed: 3002327]
13. Pettipher R. The roles of the prostaglandin D(2) receptors DP(1) and CRTH2 in promoting allergic responses. *Br J Pharmacol*. 2008; 153(Suppl 1):S191–S199. [PubMed: 17965752]
14. Spik I, Brenuchon C, Angeli V, Staumont D, Fleury S, Capron M, Trottein F, Dombrowicz D. Activation of the prostaglandin D2 receptor DP2/CRTH2 increases allergic inflammation in mouse. *J Immunol*. 2005; 174:3703–3708. [PubMed: 15749909]
15. Harris SG, Phipps RP. Prostaglandin D(2), its metabolite 15-d-PGJ(2), and peroxisome proliferator activated receptor gamma agonists induce apoptosis in transformed, but not normal, human T lineage cells. *Immunology*. 2002; 105:23–34. [PubMed: 11849312]
16. Hata AN, Breyer RM. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacol Ther*. 2004; 103:147–166. [PubMed: 15369681]
17. Bundy GL, Morton DR, Peterson DC, Nishizawa EE, Miller WL. Synthesis and platelet aggregation inhibiting activity of prostaglandin D analogues. *J Med Chem*. 1983; 26:790–799. [PubMed: 6854581]
18. Mahmu I, Smith DL, Whyte MA, Nelson JT, Cho D, Tokes LG, Alvarez R, Willis AL. On the identification and biological properties of prostaglandin J2. *Prostaglandins Leukot Med*. 1984; 16:131–146. [PubMed: 6597446]
19. Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, Lawson JA, FitzGerald GA. Role of prostacyclin in the cardiovascular response to thromboxane A2. *Science*. 2002; 296:539–541. [PubMed: 11964481]
20. Haslam RJ, Dickinson NT, Jang EK. Cyclic nucleotides and phosphodiesterases in platelets. *Thromb Haemost*. 1999; 82:412–423. [PubMed: 10605732]
21. Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res*. 2006; 99:1293–1304. [PubMed: 17158345]
22. Hui Y, Ricciotti E, Crichton I, Yu Z, Wang D, Stubbe J, Wang M, Pure E, FitzGerald GA. Targeted deletions of cyclooxygenase-2 and atherogenesis in mice. *Circulation*. 2010; 121:2654–2660. [PubMed: 20530000]
23. Sergeant S, Rahbar E, Chilton FH. Gamma-linolenic acid, Dihomo-gamma linolenic, eicosanoids and inflammatory processes. *Eur J Pharmacol*. 2016; 785:77–86. [PubMed: 27083549]

24. Lagarde M, Bernoud-Hubac N, Calzada C, Vericel E, Guichardant M. Lipidomics of essential fatty acids and oxygenated metabolites. *Mol Nutr Food Res*. 2013; 57:1347–1358. [PubMed: 23818385]
25. Needleman P, Whitaker MO, Wyche A, Watters K, Sprecher H, Raz A. Manipulation of platelet aggregation by prostaglandins and their fatty acid precursors: pharmacological basis for a therapeutic approach. *Prostaglandins*. 1980; 19:165–181. [PubMed: 6247744]
26. Negishi M, Sugimoto Y, Ichikawa A. Prostanoid receptors and their biological actions. *Prog Lipid Res*. 1993; 32:417–434. [PubMed: 8309950]
27. Kramer HJ, Stevens J, Grimminger F, Seeger W. Fish oil fatty acids and human platelets: dose-dependent decrease in dienoic and increase in trienoic thromboxane generation. *Biochem Pharmacol*. 1996; 52:1211–1217. [PubMed: 8937428]
28. Fischer S, Weber PC. Thromboxane (TX)A₃ and prostaglandin (PG)I₃ are formed in man after dietary eicosapentaenoic acid: identification and quantification by capillary gas chromatography-electron impact mass spectrometry. *Biomed Mass Spectrom*. 1985; 12:470–476. [PubMed: 2996649]
29. Wada M, DeLong CJ, Hong YH, Rieke CJ, Song I, Sidhu RS, Yuan C, Warnock M, Schmaier AH, Yokoyama C, et al. Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products. *J Biol Chem*. 2007; 282:22254–22266. [PubMed: 17519235]
30. Iyu D, Glenn JR, White AE, Johnson A, Heptinstall S, Fox SC. The role of prostanoid receptors in mediating the effects of PGE₃ on human platelet function. *Thromb Haemost*. 2012; 107:797–799. [PubMed: 22318645]
31. Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc Natl Acad Sci U S A*. 1979; 76:944–948. [PubMed: 218223]
32. Kobzar G, Mardla V, Jarving I, Samel N. Comparison of anti-aggregatory effects of PGI₂, PGI₃ and iloprost on human and rabbit platelets. *Cell Physiol Biochem*. 2001; 11:279–284. [PubMed: 11684817]
33. Hegde S, Kaushal N, Ravindra KC, Chiaro C, Hafer KT, Gandhi UH, Thompson JT, van den Heuvel JP, Kennett MJ, Hankey P, et al. Delta12-prostaglandin J₃, an omega-3 fatty acid-derived metabolite, selectively ablates leukemia stem cells in mice. *Blood*. 2011; 118:6909–6919. [PubMed: 21967980]
34. Lefils-Lacourtablaise J, Socorro M, Geloën A, Daira P, Debar C, Loizon E, Guichardant M, Dominguez Z, Vidal H, Lagarde M, et al. The eicosapentaenoic acid metabolite 15-deoxy-delta(12, 14)-prostaglandin J₃ increases adiponectin secretion by adipocytes partly via a PPARgamma-dependent mechanism. *PLoS One*. 2013; 8:e63997. [PubMed: 23734181]
35. Jin L, Lin S, Rong H, Zheng S, Jin S, Wang R, Li Y. Structural basis for iloprost as a dual peroxisome proliferator-activated receptor alpha/delta agonist. *J Biol Chem*. 2011; 286:31473–31479. [PubMed: 21775429]
36. Radmark O, Samuelsson B. 5-Lipoxygenase: mechanisms of regulation. *J Lipid Res*. 2009; 50(Suppl):S40–S45. [PubMed: 18987389]
37. Singh RK, Gupta S, Dastidar S, Ray A. Cysteinyl leukotrienes and their receptors: molecular and functional characteristics. *Pharmacology*. 2010; 85:336–349. [PubMed: 20516735]
38. Bednar M, Smith B, Pinto A, Mullane KM. Neutrophil depletion suppresses 111In-labeled platelet accumulation in infarcted myocardium. *J Cardiovasc Pharmacol*. 1985; 7:906–912. [PubMed: 2413299]
39. Knauer KA, Fish JE, Adkinson NF Jr, Lichtenstein LM, Peters SP, Newball HH. Platelet activation in antigen-induced bronchoconstriction. *N Engl J Med*. 1981; 305:892–893. [PubMed: 7278891]
40. Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, Knopf JL. A novel arachidonic acid-selective cytosolic PLA₂ contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell*. 1991; 65:1043–1051. [PubMed: 1904318]
41. Woods JW, Evans JF, Ethier D, Scott S, Vickers PJ, Hearn L, Heibin JA, Charleson S, Singer II. 5-lipoxygenase and 5-lipoxygenase-activating protein are localized in the nuclear envelope of activated human leukocytes. *J Exp Med*. 1993; 178:1935–1946. [PubMed: 8245774]

42. Brock TG, Paine R 3rd, Peters-Golden M. Localization of 5-lipoxygenase to the nucleus of unstimulated rat basophilic leukemia cells. *J Biol Chem*. 1994; 269:22059–22066. [PubMed: 8071328]
43. Dixon RA, Diehl RE, Opas E, Rands E, Vickers PJ, Evans JF, Gillard JW, Miller DK. Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature*. 1990; 343:282–284. [PubMed: 2300173]
44. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science*. 1987; 237:1171–1176. [PubMed: 2820055]
45. Sjolinder M, Tornhamre S, Claesson HE, Hydman J, Lindgren J. Characterization of a leukotriene C4 export mechanism in human platelets: possible involvement of multidrug resistance-associated protein 1. *J Lipid Res*. 1999; 40:439–446. [PubMed: 10064732]
46. Pace-Asciak CR, Klein J, Spielberg SP. Metabolism of leukotriene A4 into C4 by human platelets. *Biochim Biophys Acta*. 1986; 877:68–74. [PubMed: 2872925]
47. Sala A, Zarini S, Folco G, Murphy RC, Henson PM. Differential metabolism of exogenous and endogenous arachidon-ic acid in human neutrophils. *J Biol Chem*. 1999; 274:28264–28269. [PubMed: 10497182]
48. Penrose JF, Spector J, Lam BK, Friend DS, Xu K, Jack RM, Austen KF. Purification of human lung leukotriene C4 synthase and preparation of a polyclonal antibody. *Am J Respir Crit Care Med*. 1995; 152:283–289. [PubMed: 7599836]
49. Maugeri N, Evangelista V, Celardo A, Dell'Elba G, Martelli N, Piccardoni P, de Gaetano G, Cerletti C. Polymorphonuclear leukocyte-platelet interaction: role of P-selectin in thromboxane B2 and leukotriene C4 cooperative synthesis. *Thromb Haemost*. 1994; 72:450–456. [PubMed: 7531878]
50. Maclouf J, Antoine C, Henson PM, Murphy RC. Leukotriene C4 formation by transcellular biosynthesis. *Ann N Y Acad Sci*. 1994; 714:143–150. [PubMed: 8017763]
51. Bigby TD, Meslier N. Transcellular lipoxygenase metabolism between monocytes and platelets. *J Immunol*. 1989; 143:1948–1954. [PubMed: 2550547]
52. Laidlaw TM, Kidder MS, Bhattacharyya N, Xing W, Shen S, Milne GL, Castells MC, Chhay H, Boyce JA. Cysteinyl leukotriene overproduction in aspirin-exacerbated respiratory disease is driven by platelet-adherent leukocytes. *Blood*. 2012; 119:3790–3798. [PubMed: 22262771]
53. Laidlaw TM, Boyce JA. Platelets in patients with aspirin-exacerbated respiratory disease. *J Allergy Clin Immunol*. 2015; 135:1407–1414. quiz 1415. [PubMed: 26051947]
54. Powell WS, Gravel S, Khanapure SP, Rokach J. Biological inactivation of 5-oxo-6,8,11,14-eicosatetraenoic acid by human platelets. *Blood*. 1999; 93:1086–1096. [PubMed: 9920859]
55. Hasegawa S, Ichiyama T, Hashimoto K, Suzuki Y, Hirano R, Fukano R, Furukawa S. Functional expression of cysteinyl leukotriene receptors on human platelets. *Platelets*. 2010; 21:253–259. [PubMed: 20433311]
56. Mause SF, von Hundelshausen P, Zerneck A, Koenen RR, Weber C. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. *Arterioscler Thromb Vasc Biol*. 2005; 25:1512–1518. [PubMed: 15890969]
57. von Hundelshausen P, Koenen RR, Sack M, Mause SF, Adriaens W, Proudfoot AE, Hackeng TM, Weber C. Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. *Blood*. 2005; 105:924–930. [PubMed: 15459010]
58. Koenen RR, von Hundelshausen P, Nesmelova IV, Zerneck A, Liehn EA, Sarabi A, Kramp BK, Piccinini AM, Paludan SR, Kowalska MA, et al. Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. *Nat Med*. 2009; 15:97–103. [PubMed: 19122657]
59. von Hundelshausen P, Koenen RR, Weber C. Platelet-mediated enhancement of leukocyte adhesion. *Microcirculation*. 2009; 16:84–96. [PubMed: 19115139]
60. Cummings HE, Liu T, Feng C, Laidlaw TM, Conley PB, Kanaoka Y, Boyce JA. Cutting edge: leukotriene C4 activates mouse platelets in plasma exclusively through the type 2 cysteinyl leukotriene receptor. *J Immunol*. 2013; 191:5807–5810. [PubMed: 24244016]

61. Mais DE, Saussy DL Jr, Magee DE, Bowling NL. Interaction of 5-HETE, 12-HETE, 15-HETE and 5,12-diHETE at the human platelet thromboxane A₂/prostaglandin H₂ receptor. *Eicosanoids*. 1990; 3:121–124. [PubMed: 2169775]
62. Setty BN, Werner MH, Hannun YA, Stuart MJ. 15-Hydroxyeicosatetraenoic acid-mediated potentiation of thrombin-induced platelet functions occurs via enhanced production of phosphoinositide-derived second messengers—sn-1,2-diacylglycerol and inositol-1,4,5-trisphosphate. *Blood*. 1992; 80:2765–2773. [PubMed: 1333301]
63. Mehta P, Mehta J, Lawson D, Krop I, Letts LG. Leukotrienes potentiate the effects of epinephrine and thrombin on human platelet aggregation. *Thromb Res*. 1986; 41:731–738. [PubMed: 3008372]
64. Funk CD, Furci L, FitzGerald GA. Molecular cloning, primary structure, and expression of the human platelet/ erythroleukemia cell 12-lipoxygenase. *Proc Natl Acad Sci U S A*. 1990; 87:5638–5642. [PubMed: 2377602]
65. Funk CD, Furci L, Fitzgerald GA. Molecular cloning of the human platelet 12-lipoxygenase. *Trans Assoc Am Phys*. 1990; 103:180–186. [PubMed: 1721466]
66. Yamamoto S. Mammalian lipoxygenases: molecular structures and functions. *Biochim Biophys Acta*. 1992; 1128:117–131. [PubMed: 1420284]
67. Chang J, Blazek E, Kreft AF, Lewis AJ. Inhibition of platelet and neutrophil phospholipase A₂ by hydroxyeicosatetraenoic acids (HETES). A novel pharmacological mechanism for regulating free fatty acid release. *Biochem Pharmacol*. 1985; 34:1571–1575. [PubMed: 3994765]
68. Sekiya F, Takagi J, Sasaki K, Kawajiri K, Kobayashi Y, Sato F, Saito Y. Feedback regulation of platelet function by 12S-hydroxyeicosatetraenoic acid: inhibition of arachidonic acid liberation from phospholipids. *Biochim Biophys Acta*. 1990; 1044:165–168. [PubMed: 2111172]
69. Johnson EN, Brass LF, Funk CD. Increased platelet sensitivity to ADP in mice lacking platelet-type 12-lipoxygenase. *Proc Natl Acad Sci U S A*. 1998; 95:3100–3105. [PubMed: 9501222]
70. Takenaga M, Hirai A, Terano T, Tamura Y, Kitagawa H, Yoshida S. Comparison of the in vitro effect of eicosapentaenoic acid (EPA)-derived lipoxygenase metabolites on human platelet function with those of arachidonic acid. *Thromb Res*. 1986; 41:373–384. [PubMed: 3010490]
71. Fonlupt P, Croset M, Lagarde M. 12-HETE inhibits the binding of PGH₂/TXA₂ receptor ligands in human platelets. *Thromb Res*. 1991; 63:239–248. [PubMed: 1837628]
72. Calzada C, Vericel E, Lagarde M. Low concentrations of lipid hydroperoxides prime human platelet aggregation specifically via cyclo-oxygenase activation. *Biochem J*. 1997; 325(Pt 2):495–500. [PubMed: 9230133]
73. Coulon L, Calzada C, Moulin P, Vericel E, Lagarde M. Activation of p38 mitogen-activated protein kinase/cytosolic phospholipase A₂ cascade in hydroperoxide-stressed platelets. *Free Radic Biol Med*. 2003; 35:616–625. [PubMed: 12957654]
74. Yeung J, Apopa PL, Vesci J, Stolla M, Rai G, Simeonov A, Jadhav A, Fernandez-Perez P, Maloney DJ, Boutaud O, et al. 12-lipoxygenase activity plays an important role in PAR4 and GPVI-mediated platelet reactivity. *Thromb Haemost*. 2013; 110:569–581. [PubMed: 23784669]
75. Yeung J, Apopa PL, Vesci J, Kenyon V, Rai G, Jadhav A, Simeonov A, Holman TR, Maloney DJ, Boutaud O, et al. Protein kinase C regulation of 12-lipoxygenase-mediated human platelet activation. *Mol Pharmacol*. 2012; 81:420–430. [PubMed: 22155783]
76. Yeung J, Tourdot BE, Adili R, Green AR, Freedman CJ, Fernandez-Perez P, Yu J, Holman TR, Holinstat M. 12(S)-HETrE, a 12-lipoxygenase oxylipin of dihomogamma-linolenic acid, inhibits thrombosis via Galphas signaling in platelets. *Arterioscler Thromb Vasc Biol*. 2016; 36:2068–2077. [PubMed: 27470510]
77. Yeung J, Tourdot BE, Fernandez-Perez P, Vesci J, Ren J, Smyrniotis CJ, Luci DK, Jadhav A, Simeonov A, Maloney DJ, et al. Platelet 12-LOX is essential for FcγRIIa-mediated platelet activation. *Blood*. 2014; 124:2271–2279. [PubMed: 25100742]
78. Thomas CP, Morgan LT, Maskrey BH, Murphy RC, Kuhn H, Hazen SL, Goodall AH, Hamali HA, Collins PW, O'Donnell VB. Phospholipid-esterified eicosanoids are generated in agonist-activated human platelets and enhance tissue factor-dependent thrombin generation. *J Biol Chem*. 2010; 285:6891–6903. [PubMed: 20061396]

79. Guo Y, Zhang W, Giroux C, Cai Y, Ekambaram P, Dilly AK, Hsu A, Zhou S, Maddipati KR, Liu J, et al. Identification of the orphan G protein-coupled receptor GPR31 as a receptor for 12-(S)-hydroxyeicosatetraenoic acid. *J Biol Chem*. 2011; 286:33832–33840. [PubMed: 21712392]
80. Hampson AJ, Grimaldi M. 12-hydroxyeicosatetraenoate (12-HETE) attenuates AMPA receptor-mediated neurotoxicity: evidence for a G-protein-coupled HETE receptor. *J Neurosci*. 2002; 22:257–264. [PubMed: 11756509]
81. Sun L, Xu YW, Han J, Liang H, Wang N, Cheng Y. 12/15-Lipoxygenase metabolites of arachidonic acid activate PPARgamma: a possible neuroprotective effect in ischemic brain. *J Lipid Res*. 2015; 56:502–514. [PubMed: 25605873]
82. Ikei KN, Yeung J, Apopa PL, Ceja J, Vescei J, Holman TR, Holinstat M. Investigations of human platelet-type 12-lipoxygenase: role of lipoxygenase products in platelet activation. *J Lipid Res*. 2012; 53:2546–2559. [PubMed: 22984144]
83. Fischer S, von Schacky C, Siess W, Strasser T, Weber PC. Uptake, release and metabolism of docosahexaenoic acid (DHA, c22:6 omega 3) in human platelets and neutrophils. *Biochem Biophys Res Commun*. 1984; 120:907–918. [PubMed: 6329189]
84. Akiba S, Murata T, Kitatani K, Sato T. Involvement of lipoxygenase pathway in docosapentaenoic acid-induced inhibition of platelet aggregation. *Biol Pharm Bull*. 2000; 23:1293–1297. [PubMed: 11085354]
85. Careaga MM, Sprecher H. Synthesis of two hydroxy fatty acids from 7,10,13,16,19-docosapentaenoic acid by human platelets. *J Biol Chem*. 1984; 259:14413–14417. [PubMed: 6438096]
86. Yeung J., Tourdot, BE., Adili, R., Green, AR., Freedman, CJ., Fernandez-Perez, P., Yu, J., Holman, TR., Holinstat, M. 12-HETrE, a 12-lipoxygenase oxylipin of dihomo-gamma-linolenic acid. Inhibits Thrombosis via Galphas Signaling in Platelets. *Arterioscler Thromb Vasc Biol*. 2016. doi:[10.1161/ATVBAHA.116.308050](https://doi.org/10.1161/ATVBAHA.116.308050)
87. Brash AR, Boeglin WE, Chang MS. Discovery of a second 15S-lipoxygenase in humans. *Proc Natl Acad Sci U S A*. 1997; 94:6148–6152. [PubMed: 9177185]
88. Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR. 15-lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. *Am J Pathol*. 1999; 155:235–245. [PubMed: 10393855]
89. Ivanov I, Kuhn H, Heydeck D. Structural and functional biology of arachidonic acid 15-lipoxygenase-1 (ALOX15). *Gene*. 2015; 573:1–32. [PubMed: 26216303]
90. Jiang WG, Watkins G, Douglas-Jones A, Mansel RE. Reduction of isoforms of 15-lipoxygenase (15-LOX)-1 and 15-LOX-2 in human breast cancer. *Prostaglandins Leukot Essent Fatty Acids*. 2006; 74:235–245. [PubMed: 16556493]
91. Shureiqi I, Wu Y, Chen D, Yang XL, Guan B, Morris JS, Yang P, Newman RA, Broaddus R, Hamilton SR, et al. The critical role of 15-lipoxygenase-1 in colorectal epithelial cell terminal differentiation and tumorigenesis. *Cancer Res*. 2005; 65:11486–11492. [PubMed: 16357157]
92. Wong PY, Westlund P, Hamberg M, Granstrom E, Chao PH, Samuelsson B. 15-Lipoxygenase in human platelets. *J Biol Chem*. 1985; 260:9162–9165. [PubMed: 3926763]
93. Kim HY, Karanian JW, Salem N Jr. Formation of 15-lipoxygenase product from docosahexaenoic acid (22:6w3) by human platelets. *Prostaglandins*. 1990; 40:539–549. [PubMed: 2147774]
94. Vericel E, Lagarde M. 15-Hydroperoxyeicosatetraenoic acid inhibits human platelet aggregation. *Lipids*. 1980; 15:472–474. [PubMed: 7401944]
95. Vedelago HR, Mahadevappa VG. Differential effects of 15-HPETE on arachidonic acid metabolism in collagen-stimulated human platelets. *Biochem Biophys Res Commun*. 1988; 150:177–184. [PubMed: 3122753]
96. Bild G, Bhat S, Axelrod B, Iatridis P. Inhibition of aggregation of human platelets by 8, 15-dihydroperoxides of 5, 9, 11, 13-eicosatetraenoic and 9, 11, 13-eicosatrienoic acids. *Prostaglandins*. 1978; 16:795–801.
97. Vijil C, Hermansson C, Jeppsson A, Bergstrom G, Hulten LM. Arachidonate 15-lipoxygenase enzyme products increase platelet aggregation and thrombin generation. *PLoS One*. 2014; 9:e88546. [PubMed: 24533104]

98. Lannan, KL., Spinelli, SL., Blumberg, N., Phipps, RP. Maresin 1 induces a novel pro-resolving phenotype in human platelets. *J Thromb Haemost.* 2017. doi:[10.1111/jth.13620](https://doi.org/10.1111/jth.13620)
99. Yamaja Setty BN, Berger M, Stuart MJ. 13-Hydroxyoctadeca-9,11-dienoic acid (13-HODE) inhibits thromboxane A2 synthesis, and stimulates 12-HETE production in human platelets. *Biochem Biophys Res Commun.* 1987; 148:528–533. [PubMed: 3120708]
100. Guichardant M, Naltachayan-Durbin S, Lagarde M. Occurrence of the 15-hydroxy derivative of dihomogammalinolenic acid in human platelets and its biological effect. *Biochim Biophys Acta.* 1988; 962:149–154. [PubMed: 2843240]
101. Tloti MA, Moon DG, Weston LK, Kaplan JE. Effect of 13-hydroxyoctadeca-9,11-dienoic acid (13-HODE) on thrombin induced platelet adherence to endothelial cells in vitro. *Thromb Res.* 1991; 62:305–317. [PubMed: 1866713]
102. Chen P, Vericel E, Lagarde M, Guichardant M. Poxyrins, a class of oxygenated products from polyunsaturated fatty acids, potentially inhibit blood platelet aggregation. *FASEB J.* 2011; 25:382–388. [PubMed: 20833872]
103. Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, Nebert DW. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics.* 2004; 14:1–18. [PubMed: 15128046]
104. Wu S, Moomaw CR, Tomer KB, Falck JR, Zeldin DC. Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *J Biol Chem.* 1996; 271:3460–3468. [PubMed: 8631948]
105. Capdevila JH, Falck JR, Harris RC. Cytochrome P450 and arachidonic acid bioactivation. Molecular and functional properties of the arachidonate monooxygenase. *J Lipid Res.* 2000; 41:163–181. [PubMed: 10681399]
106. Zhu Y, Schieber EB, McGiff JC, Balazy M. Identification of arachidonate P-450 metabolites in human platelet phospholipids. *Hypertension.* 1995; 25:854–859. [PubMed: 7721444]
107. Fitzpatrick FA, Ennis MD, Baze ME, Wynalda MA, McGee JE, Liggett WF. Inhibition of cyclooxygenase activity and platelet aggregation by epoxyeicosatrienoic acids. Influence of stereochemistry. *J Biol Chem.* 1986; 261:15334–15338. [PubMed: 3095326]
108. Balazy M. Metabolism of 5,6-epoxyeicosatrienoic acid by the human platelet. Formation of novel thromboxane analogs. *J Biol Chem.* 1991; 266:23561–23567. [PubMed: 1748634]
109. VanRollins M. Epoxygenase metabolites of docosahexaenoic and eicosapentaenoic acids inhibit platelet aggregation at concentrations below those affecting thromboxane synthesis. *J Pharmacol Exp Ther.* 1995; 274:798–804. [PubMed: 7636743]
110. Krotz F, Riexinger T, Buerkle MA, Nithipatikom K, Gloe T, Sohn HY, Campbell WB, Pohl U. Membrane-potential-dependent inhibition of platelet adhesion to endothelial cells by epoxyeicosatrienoic acids. *Arterioscler Thromb Vasc Biol.* 2004; 24:595–600. [PubMed: 14715644]
111. Krotz F, Hellwig N, Burkle MA, Lehrer S, Riexinger T, Mannell H, Sohn HY, Klauss V, Pohl U. A sulfaphenazole-sensitive EDHF opposes platelet-endothelium interactions in vitro and in the hamster microcirculation in vivo. *Cardiovasc Res.* 2010; 85:542–550. [PubMed: 19717402]
112. Tunaru S, Chennupati R, Nusing RM, Offermanns S. Arachidonic acid metabolite 19(S)-HETE induces vasorelaxation and platelet inhibition by activating prostacyclin (IP) receptor. *PLoS One.* 2016; 11:e0163633. [PubMed: 27662627]
113. Hill E, Fitzpatrick F, Murphy RC. Biological activity and metabolism of 20-hydroxyeicosatetraenoic acid in the human platelet. *Br J Pharmacol.* 1992; 106:267–274. [PubMed: 1327375]
114. Schwartzman ML, Falck JR, Yadagiri P, Escalante B. Metabolism of 20-hydroxyeicosatetraenoic acid by cyclooxygenase. Formation and identification of novel endothelium-dependent vasoconstrictor metabolites. *J Biol Chem.* 1989; 264:11658–11662. [PubMed: 2501294]
115. Tsai IJ, Croft KD, Puddey IB, Beilin LJ, Barden A. 20-Hydroxyeicosatetraenoic acid synthesis is increased in human neutrophils and platelets by angiotensin II and endothelin-1. *Am J Physiol Heart Circ Physiol.* 2011; 300:H1194–H1200. [PubMed: 21239640]

116. Knapp HR, Miller AJ, Lawson JA. Urinary excretion of diols derived from eicosapentaenoic acid during n-3 fatty acid ingestion by man. *Prostaglandins*. 1991; 42:47–54. [PubMed: 1771239]
117. Fleming I. Cytochrome p450 and vascular homeostasis. *Circ Res*. 2001; 89:753–762. [PubMed: 11679404]
118. VanRollins M, Kaduce TL, Fang X, Knapp HR, Spector AA. Arachidonic acid diols produced by cytochrome P-450 monooxygenases are incorporated into phospholipids of vascular endothelial cells. *J Biol Chem*. 1996; 271:14001–14009. [PubMed: 8662855]
119. Kim DH, Puri N, Sodhi K, Falck JR, Abraham NG, Shapiro J, Schwartzman ML. Cyclooxygenase-2 dependent metabolism of 20-HETE increases adiposity and adipocyte enlargement in mesenchymal stem cell-derived adipocytes. *J Lipid Res*. 2013; 54:786–793. [PubMed: 23293373]
120. Pratt PF, Rosolowsky M, Campbell WB. Effects of epoxyeicosatrienoic acids on polymorphonuclear leukocyte function. *Life Sci*. 2002; 70:2521–2533. [PubMed: 12173415]
121. Recchiuti A, Serhan CN. Pro-resolving lipid mediators (SPMs) and their actions in regulating miRNA in novel resolution circuits in inflammation. *Front Immunol*. 2012; 3:298. [PubMed: 23093949]
122. Levy BD, Romano M, Chapman HA, Reilly JJ, Drazen J, Serhan CN. Human alveolar macrophages have 15-lipoxygenase and generate 15(S)-hydroxy-5,8,11-cis-13-trans-eicosatetraenoic acid and lipoxins. *J Clin Invest*. 1993; 92:1572–1579. [PubMed: 8376607]
123. Chavis C, Vachier I, Chanez P, Bousquet J, Godard P. 5(S), 15(S)-dihydroxyeicosatetraenoic acid and lipoxin generation in human polymorphonuclear cells: dual specificity of 5-lipoxygenase towards endogenous and exogenous precursors. *J Exp Med*. 1996; 183:1633–1643. [PubMed: 8666921]
124. Serhan CN, Sheppard KA. Lipoxin formation during human neutrophil-platelet interactions. Evidence for the transformation of leukotriene A4 by platelet 12-lipoxygenase in vitro. *J Clin Invest*. 1990; 85:772–780. [PubMed: 2155925]
125. Edenius C, Stenke L, Lindgren JA. On the mechanism of transcellular lipoxin formation in human platelets and granulocytes. *Eur J Biochem*. 1991; 199:401–409. [PubMed: 1906402]
126. Borgeson E, Docherty NG, Murphy M, Rodgers K, Ryan A, O'Sullivan TP, Guiry PJ, Goldschmeding R, Higgins DF, Godson C. Lipoxin A(4) and benzo-lipoxin A(4) attenuate experimental renal fibrosis. *FASEB J*. 2011; 25:2967–2979. [PubMed: 21628447]
127. Vital SA, Becker F, Holloway PM, Russell J, Perretti M, Granger DN, Gavins FN. Formyl-peptide receptor 2/3/lipoxin A4 receptor regulates neutrophil-platelet aggregation and attenuates cerebral inflammation: impact for therapy in cardiovascular disease. *Circulation*. 2016; 133:2169–2179. [PubMed: 27154726]
128. Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN. Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. *Blood*. 2008; 112:848–855. [PubMed: 18480426]
129. Fredman G, Van Dyke TE, Serhan CN. Resolvin E1 regulates adenosine diphosphate activation of human platelets. *Arterioscler Thromb Vasc Biol*. 2010; 30:2005–2013. [PubMed: 20702811]
130. Abdulnour RE, Dalli J, Colby JK, Krishnamoorthy N, Timmons JY, Tan SH, Colas RA, Petasis NA, Serhan CN, Levy BD. Maresin 1 biosynthesis during platelet-neutrophil interactions is organ-protective. *Proc Natl Acad Sci U S A*. 2014; 111:16526–16531. [PubMed: 25369934]
131. Lagarde M, Vericel E, Liu M, Chen P, Guichardant M. Structure-function relationships of non-cyclic dioxygenase products from polyunsaturated fatty acids: poxytrins as a class of bioactive derivatives. *Biochimie*. 2014; 107(Pt A):91–94. [PubMed: 25223888]
132. Balas L, Guichardant M, Durand T, Lagarde M. Confusion between protectin D1 (PD1) and its isomer protectin DX (PDX). An overview on the dihydroxy-docosatrienes described to date. *Biochimie*. 2014; 99:1–7. [PubMed: 24262603]
133. Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J Biol Chem*. 2003; 278:14677–14687. [PubMed: 12590139]

134. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc Natl Acad Sci U S A*. 2004; 101:8491–8496. [PubMed: 15152078]

Fig. 1.

Polyunsaturated fatty acids (PUFAs) are released from the embedded phospholipid bilayer membrane, which are then converted by intracellular oxygenases (COX, LOX, or CYP450) to generate wide array of oxylipins that can diffuse across the cellular membrane to be further converted by oxygenases, act on intracellular signaling component, peroxisome proliferator-activated receptor (PPAR), or act on receptor to regulate platelet function

Fig. 2.

Arachidonic acid (AA) is oxidized by 5-LOX, 12-LOX, 15-LOX, COX, and CYP450 into their respective classes of oxylipins: 1) non-leukotrienes (LTs) (5-HETE, 5-oxo-EETE) or cysteinyl-free LTs (LTA₄, LTB₄) and cysteinyl-LTs (LTC₄, LTD₄, LTE₄); 2) 12-HETE; 3) series 2 prostaglandins (PGD₂, PGE₂, PGI₂, and TXA₂); 4) epoxyeicosatrienoic acids (EETs) (5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET) and hydroxylates (19-HETE, 20-HETE)

Fig. 3. PUFA oxidation by oxygenases. **a** Dihomo- γ -linolenic acid (DGLA) is oxidized by 12-LOX, 15-LOX, and COX into the corresponding metabolites: 12-HETrE, 15-HETrE, and series 1 prostaglandins (PGD₁, PGE₁, TXA₂). **b** Docosahexaenoic acid (DHA) is also metabolized by the oxygenases into the following: 11- or 14-HDHA by 12-LOX, 17-HDHA by 15-LOX, and epoxydocosapentaenoic acids (EDPs) (7,8-EDP, 10,11-EDP, 13,14-EDP, 16,17-EDP, 19,20-EDP) by CYP450 isoforms

Fig. 4. PUFA oxidation by oxygenases. **a** 12-LOX acts on docosapentaenoic acid (DPA) to convert to 11- or 14-HDPA. **b** Linoleic acid (LA) is metabolized by 15-LOX to generate 13-HODE. **c** Eicosapentaenoic acid (EPA) is oxidized by 12-LOX, COX, CYP 450 to 12-HEPE, series 3 prostaglandins (PGD₃, PGE₃, PGI₃, and TXA₃), and epoxyeicosatetraenoic acids (EEQs) (8,9-EEQ, 11,12-EEQ, 14,15-EEQ, and 17,18-EEQ)

Fig. 5. Specialized pro-resolving lipid mediators (SPMs) constitute a wide array of lipids classes derived from the interplay of oxygenase activity on AA, EPA, and DHA. Lipoxins, (LXA_4 , LXB_4) and E series resolvin (RvE_1) are derived from AA and EPA, respectively. DHA can be indirectly converted by the interaction of the oxygenases into $MaR1$, PDX , or D series resolvin, RvD_1 .

Fig. 6.

LOX, COX, CYP 450 derived lipid mediators, and SPMs can be divided into either pro- or anti-thrombotic classes based on their effects on platelet function

Table 1

Oxylin regulation of platelets

Oxygenase	PUFA	Oxylin	Actions
COX	AA	PGD ₂	Inhibits platelet activation via DP receptor and possibly PPAR
	AA	PGE ₂	Exhibits both anti-platelet and pro-platelet activation, depending on concentrations and binding to receptors EP ₁ -EP ₄
	AA	PGI ₂	Inhibits platelet function via IP receptor and PPAR
	AA	TXA ₂	Activates platelet function via TPα receptor
	DGLA	PGD ₁	Inhibits platelet function via EP ₂ , EP ₄ and IP receptors
	DGLA	PGE ₁	Inhibits platelet function via EP ₂ , EP ₄ and IP receptors
	DGLA	TXA ₁	Inhibits platelet function via EP ₂ , EP ₄ and IP receptors
	EPA	PGD ₃	Inhibits platelet function via DP receptor and PPAR
	EPA	PGE ₃	Inhibits platelet function via EP ₂ and P ₄ receptors
	EPA	PGI ₃	Inhibits platelet function via IP receptor and PPAR
	EPA	TXA ₃	Inhibits platelet function via EP ₂ and EP ₄ receptors
5-LOX	AA	5-HETE	Exhibits both anti-platelet and pro-platelet activation; unknown mechanism of action
	AA	LTA ₄	Unknown function
	AA	LTB ₄	Unknown function
	AA	LTC ₄	Activates platelet function via CysLT ₂ R
	AA	LTD ₄	Potential of platelet activation; unknown mechanism of action
	AA	LTE ₄	Potential of platelet activation; unknown mechanism of action
	AA	5-oxo-EETE	Unknown function
12-LOX	AA	12-HETE	Exhibits both anti-platelet and pro-platelet activation possibly through 12-HETER
	DGLA	12-HETrE	Inhibits platelet through Gα _s
	DHA	11-HDHA	Inhibits platelet activation; unknown mechanism of action
	DHA	14-HDHA	Inhibits platelet activation; unknown mechanism of action
	DPA	11-HDPA	Inhibits platelet activation possibly through antagonism of COX-1
	DPA	14-HDPA	Inhibits platelet activation possibly through antagonism of COX-1
	EPA	12-HEPE	Inhibits platelet activation; unknown mechanism of action
15-LOX	AA	15-HETE	Exhibits both anti-platelet and pro-platelet activation; unknown mechanism of action
	DGLA	15-HETrE	Exhibits both anti-platelet and pro-platelet activation; unknown mechanism of action
	DHA	17-DHDA	Exhibits both anti-platelet and pro-platelet activation; unknown mechanism of action
	DPA	13-HODE	Exhibits both anti-platelet and pro-platelet activation; unknown mechanism of action
CYP450	AA	5,6-EET	Inhibits platelet activation; unknown mechanism of action
	AA	8,9-EET	Inhibits platelet activation; unknown mechanism of action
	AA	11,12-EET	Inhibits platelet activation; unknown mechanism of action
	AA	14,15-EET	Inhibits platelet activation; unknown mechanism of action
	AA	19-HETE	Inhibits platelet function via IP receptor
	AA	20-HETE	Inhibits human platelet activation; antagonism of PGH ₂ /TXA ₂ receptor or further oxidation to inactive metabolites
	DHA	7,8-EDP	Inhibits platelet activation; unknown mechanism of action
	DHA	10,11-EDP	Inhibits platelet activation; unknown mechanism of action

Oxygenase	PUFA	Oxylipin	Actions
	DHA	13,14-EDP	Inhibits platelet activation; unknown mechanism of action
	DHA	16,17-EDP	Inhibits platelet activation; unknown mechanism of action
	DHA	19,20-EDP	Inhibits platelet activation; unknown mechanism of action
	EPA	8,9-EEQ	Inhibits platelet activation; unknown mechanism of action
	EPA	11,12-EEQ	Inhibits platelet activation; unknown mechanism of action
	EPA	14,15-EEQ	Inhibits platelet activation; unknown mechanism of action
	EPA	17,18-EEQ	Inhibits platelet activation; unknown mechanism of action
5-LOX, 15-LOX	DHA	Resolvin D ₁	Potentiation of platelet activation possibly through GPR32
12-LOX	DHA	MaR1	Exhibits both anti-platelet and pro-platelet activation; unknown mechanism of action
15-LOX, 5-LOX	DHA	Protectin DX	Inhibits platelet activation; unknown mechanism of action
COX-2, 5-LOX, CYP450	EPA	Resolvin E ₁	Inhibits platelet activation possibly through ChemR23
15-LOX, 5-LOX, 12-LOX	AA	LXA ₄	Modulate neutrophil-platelet aggregation through LXA ₄ receptor; however unknown direct effect on platelet function and mechanism of action
	AA	LXB ₄	Unknown function