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12-Lipoxygenase: A Potential Target for Novel Anti-Platelet Therapeutics

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Abstract

Platelets play an essential role in the regulation of hemostasis and thrombosis and controlling their level of activation is central to prevention of occlusive clot formation and stroke. Although a number of anti-platelet targets have been identified to address this issue including COX-1, the $P2Y_{12}$ receptor, the integrin αIIbβ3, and more recently the protease-activated receptor-1, these targets often result in a significant increased risk of bleeding which may lead to pathologies as serious as the thrombosis they were meant to treat including intracranial hemorrhage and gastrointestinal bleeding. Therefore, alternative approaches to treat uncontrolled platelet activation are warranted. Platelet-type 12-lipoxygenase is an enzyme which oxidizes the free fatty acid in the platelet resulting in the production of the stable metabolite 12-hydroxyeicosatetraenoic acid (12-HETE). The role of 12-HETE in the platelet has been controversial with reports associating its function as being both anti- and pro-thrombotic. In this review, the role of 12-lipoxygenase and its bioactive metabolites in regulation of platelet reactivity, clot formation, and hemostasis is described. Understanding the mechanisms by which 12-lipoxygenase and its metabolites modulate platelet function may lead to the development of a novel class of anti-platelet therapies targeting the enzyme in order to attenuate injury-induced clot formation, vessel occlusion and pathophysiological shifts in hemostasis.

Keywords

12-LOX; anti-platelet therapeutics; eicosanoids; fatty acid oxidation; hemostasis; lipoxygenase; platelets; thrombosis

PLATELET THERAPY IN CARDIOVASCULAR DISEASE

Incidence of CVD

Recently, the American Heart Association reported that cardiovascular diseases (CVD) accounted for 33.6% of all deaths in the United States [1], and is the single leading cause of death in the nation and worldwide [2] occurring before age 65. Furthermore, more than 67.3% of adults over the age of 20 are reported to be obese, which is one of the major contributing factors to developing CVD along with smoking. Consequently, surgical procedures for cardiovascular complications have increased by 27% from 1997 to 2007 [1]. Despite these alarming statistics, the mortality rate resulting from CVD complications has

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slightly decreased, although the burden of the disease still remains high. This is due, in part, to the successful development and widespread use in the clinic of a number of anti-platelet drugs.

Current Anti-Platelet Drugs

One of the earliest targets for anti-platelet therapy was cyclooxygenase-1 (COX-1), responsible for the formation of prostanoids, which are involved in inflammation [3]. A number of pharmacological approaches have been developed over the years to inhibit COX activation in platelets including aspirin, indomethacin, and nonsteroidal anti-inflammatory drugs (NSAIDS) [4]. The efficacy in treating and preventing vascular-occulsive events with COX-1 inhibitors may be limiting in a segment of the population [5, 6]. One study for example, reported that greater than 33% of the patients with CVD exhibited aspirin resistance [3, 7]. These observations however are controversial since no generally acceptable definition of aspirin resistance exists and intra-individual differences in COX-1 inhibition can be highly variable [8]. Another target on the platelet which has successfully been inhibited in order to limit platelet activation is the ADP receptor, $P2Y_{12}$. Targeting $P2Y_{12}$ with clopidogrel, a pro-drug that binds to and inhibits receptor activation, has been shown to be effective in further reducing platelet activation when given as dual anti-platelet therapy with aspirin [9]. However, due to the nature of this pro-drug and the genetic variability of the cytochrome P450 enzyme required to convert clopidogrel to its active form, its utility is limited as is evident from the development of next generation $P2Y_{12}$ inhibitors ticagrelor and prasugrel [10, 11]. Integrin IIb/IIIa inhibitors (abciximab, eptifibatide, tirofiban), although a breakthrough in antibody-based anti-platelet therapy [11-13], have resulted in an increase in bleeding complications following PCI. Furthermore, some patients developed frequent myocardial infarction and refractory ischemia post-tibrofiban administration [14].

Adverse side effects such as bleeding are of primary concern for current treatment against platelet activation in CVD therapy, especially prior to and during surgical procedures [15]. Thus, alternative strategies which would inhibit platelet activation while minimizing the bleeding side effect are warranted. A novel target for anti-platelet therapy in the treatment of patients with cardiovascular related diseases may be human 12-lipoxygenase (12-LOX). In order to develop potential 12-LOX inhibitors however, we must have a comprehensive understanding of its pathophysiological and biochemical implications and the role 12-LOX metabolites play in the vascular system. Our current knowledge of this enzyme and its oxidized products in platelets and other tissues is still limited, but the preliminary evidence shows the potential advantages of inhibiting 12-LOX on the platelet to treating human diseases.

Historical Overview of Lipoxygenases

Lipoxygenases (LOs) are a family of nonheme iron dioxygenases, originally known as lipoxidases that catalyze the oxidation of polyunsaturated fatty-acids such as linoleic acid or arachidonic acid (AA) containing the *cis*-methylene interrupted diene structure and esters yielding conjugated hydroperoxides [16]. The earliest lipoxygenase studies showed 15-lipoxygenase purified from soybean oxidized unsaturated moieties of fatty acids [17] at a specific carbon site of the acyl chain; that eventually allowed lipoxygenase isoforms to be identified according to their site of carbon oxidation and stereochemistry. The transformation of AA to 12(S)-hydroxy-5,8,10,14-eicosatetraenoic acid (12(S)-HETE) was first demonstrated in human and bovine platelets in the mid-1970s [18]. These metabolites, produced by 5-, 8 -, 12-, and 15- LO, have been reported to potentially form signaling lipid mediators that exert their effects through the G protein–coupled receptor (GPCR) [19]. Lipoxygenases and their metabolites have been demonstrated experimentally and clinically to be restricted to specific cells or tissues and show significant species specificity. To date,

three isoforms of 12(S)-lipoxygenases have been identified in human accordingly to their cell type: epidermis, leukocyte, and platelet [20]. 12-LOX activation has been reported to play a role in a number of diseases including psoriasis [21] and ulcerative colitis [22, 23]. Despite intensive research in this field, the majority of the discoveries of 12-lipoxygenase functions and their implications have been established using animal models. Thus, our current understanding of the biological significance of these enzymes and products in humans is still limited. 12-LOX isoforms and their products will be the focus of this review showing their involvement in platelet reactivity contributing to hemostasis and thrombosis and the overall scheme in developing alternative targets to prevent vascular occlusion, myocardial infarction, and stroke.

OVERVIEW of 12-LOX

Activation of Platelets through a number of receptors is known to result in activation of cytosolic phospholipase A_2 ($cPLA_2$), a lipid lipase that generates free fatty acid such as AA from the phospholipid membrane by cleaving the sn-2 position [18]. Once AA is formed, it is available for oxidation by either COX-1 or 12-LOX and can produce a number of bioactive lipid metabolites [18]. In leukocytes, oxidized AA will result in the production of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) [24, 25] as pro-inflammatory effectors or thromboxane (TxA_2) and 12-HETE in platelets. Recently, we have shown that COX-1 and 12-LOX-mediated signaling may rely on different pools of AA based on the kinetics of TxA_2 and 12-HETE formation and their differential reliance on $cPLA_2$ at the surface of the platelet [26]. 12-LOX activation is also thought to be important for dense granule secretion in platelets as well as normal platelet aggregation and adhesion [27]**.** This is not surprising considering blocking 12-LOX attenuates aggregation and integrin activation in the presence of thromboxane, collagen, thrombin, and protease-activated receptors (PARs) [28-30]. Additionally, 12-LOX has also been implicated to play a role in regulating calcium mobilization. A role for 12-LOX in the platelet using one of the classical 12-LOX inhibitors, baicalein, was first described in the mid-1990s, where stimulation with AA in the absence of 12-LOX resulted in a significant attenuation of thrombin-induced calcium $[Ca^{2+}]_i$ transients and aggregation [27]. In addition to oxidation, 12-LOX in platelets has also been reported to have lipoxin synthase activity. Lipoxins, such as LXA_4 and LXB_4, are tetraene-containing eicosanoids generated from exogenous LTA_4 that induces vasoconstriction of the smooth muscles and regulates neutrophil function *via* binding at specific recognition sites [31-34]. The molecular observations above confirm an important role for 12-LOX in human platelet reactivity and a renewed interest in this field attests to the therapeutic potential inherent with regulation of 12-LOX. Finally, although human studies to date are limited to *ex vivo* platelet reactivity and thrombosis, a number of animal models have added crucial information as to the potential role of 12-LOX in hemostasis including studies with 12/15-LO knockout mice, canines, porcine, and rabbits, show varying and sometimes unrelated physiological effects compared with humans [35] (Table 1). Although 12-LO targets and functions appear to be species related, 12-LO activation in a number of platelet models has been correlated to modulation of platelet reactivity *in vivo* (see Table 1). We must be careful not to interpret these studies to mean that 12-LOX is essential for normal platelet activation, but rather that elimination of 12-LOX protein or activity may be related to normal regulation of hemostasis and thrombosis. This is an area that will need further investigation in order to determine how the animal models translate to platelet function in the human. Recent work, however, does indicate that altered 12-LOX function may be related to defects in hemostasis *in vivo* [36].

12-LOX SUBSTRATES

Polyunsaturated Fatty Acids (PUFA) as Regulators of 12-LOX-Mediated Activity

A possible alternative approach to anti-platelet therapy may lie in the dietary intake of certain essential fatty acids (EFA). Large prospective studies have shown that there is a positive relationship between increased dietary intake of ω-3 fatty acids and reduced CVD. For instance, Eskimos from the West Coast of Greenland whose diet included large quantities of whale, fish, and seal, had a positive correlation with lower incidence of myocardial infarction, stroke, and mild tendency to bruise compared to the overall population [52]. Further, rats receiving Thomas-Hartroft thrombogenic diet were subsequently fed with unsaturated fatty acid supplements which comparatively showed reduced thromboplastin generation and mortality compared to control rats [53]. Interestingly, the incidence of mortality due to ischemic heart and cerebrovascular disease between 1950 and 1982 was found to be lower in fishing communities relative to farming villages in Japan [54-56]. This epidemiological study correlated the low level of CVD-related events with high levels of eicosapentaenoic acid (EPA) from fish. Thus, larger amount of specific PUFAs in the diet may result in an increased pool of fatty acids made available to 12-LOX in the platelet. Depending on the specific fatty acid substrates available, oxidation by 12-LOX may generate several pools of eicosanoids which may in turn act as key regulators of platelet reactivity. These nutritional studies, although supportive of a role for 12-LOX in mediating changes based on altered free fatty acid oxidation, lack the scientific rigor required in order to connect the observed benefit of PUFA supplementation with a substantive link to altered 12-LOX oxidized metabolites as being causative to these reported cardiovascular benefits. Furthermore, while the addition of PUFAs as a dietary supplement may be beneficial, the amount and type of fatty acids added to the diet needs to be carefully monitored in patients with existing cardiovascular risk. Previous studies for example, have suggested that docosahexaenoic acid (DHA) has an adverse effect on diabetics and elderly who are already suffering from a lower antioxidant potential. In one study, platelets from an elderly subject with low DHA were given low DHA supplements and exhibited lower lipid peroxidation activity, whereas incubated with high DHA concentration induced higher 12-HETE formation [57, 58]. As for EPA, fatty acid supplements given to patients resulted in attenuation of platelet aggregation. TxB_2 production has also been shown to be inhibited in platelets pre-treated with EPA [57].

12-LOX Substrate Availability

Dietary intake of certain EFAs (ω-3 or ω-6) have been shown to impact the type of metabolic products generated by 12-LOX. For instance AA, an ω-6 series fatty acid derived from cis-linoleic acid, can be found mostly in peanut oil. As for ω-3, fish oil, flaxeed, and algal oil are the common sources for α-linolenic acid (ALA), DHA, and EPA [59]. Fatty acids in platelet membranes containing DHA have been reported to be oxidized by 12-LOX to various hydroxyl docosahexaenoic acid (HDoHE) isoforms, but largely forming 14-HDoHE in an agonist and calcium-dependent manner [18]. A number of metabolic products are formed following 12-LOX oxidation of another fatty acid, EPA, which include AA, TxB_3, 12(S)-hydroxyeicosapentanoic acid (12-HEPE), and hydroxy-5,8,10-14-heptadecatetraenoic acid (HhTE) [60, 61]. One study showed that EPA conversion to TxB_3 depended on the presence of hydroxy-5,8,10, 14-eicosatetraenoic acid (12-HpETE [61], whereas, pre-incubation with 12-HETE did not induce EPA metabolism. In another case, pre-incubation with 12(S)-HpETE has been shown to increase the amount of nonesterified AA in collagen stimulated platelets, significantly enhancing platelet aggregation and the formation of TXB_2 [62]. The eicosanoid metabolites, once formed, can have a number of regulatory functions in the platelet probably through both autocrine as well as paracrine

signaling schemes. The physiological and cellular effects due to oxidized products are further discussed in detail below.

Direct 12-LOX Regulation of Eicosanoid Metabolites

Eicosanoid metabolites are able to be further oxidized by 12-LOX. 12-LOX can catalyze 5-HETE to generate 5(S), 12-dihydroxyeicosatetraenoic acid (12(S)-DHETE) and 15-HETE to 14,15-DHETE in platelets under conditions of prolonged exposure to the enzyme [63]. Similarly, exogenous AA and 5-HETE show reduced production of 5(S), 12(S)-DHETE. 11,12-DHETE has also been reported to be generated in a 12-LOX dependent manner [64].

BIOLOGICAL ROLE OF METABOLITES

Metabolite Profiling in the Blood

Profiling of lipid metabolites and mediators in whole blood in the presence of the calcium ionophore A23187, demonstrated that 12-LOX contributes a large proportion of the total products formed [65] compared with the other lipoxygenases. The predominant products were 12-HETE, 12-HEPE, and 12-hydroxydocosahexaenoic acid (12-HDHA). A significant number of 5-LOX products, including LTB_4, LTB_5, and PGE_2, were also found in circulation under these conditions. Shifting ω-3 or ω-6 content in the lipid bilayer of the cells has been shown to contribute to differences in their respective lipid mediators and metabolites and thus regulate biochemical and physiological events in the cells of the individuals [60, 66].

12-LOX Metabolite Regulation of Various Tissues

12-LOX oxidation of various fatty acids in the platelet can result in the formation of a number of unique eicosanoid metabolites. Many, if not all, of these eicosanoids can play a regulatory role both within the platelet itself as well as at distal tissues and organ systems. Previous studies support a pro-inflammatory role for eicosanoid formed through 12-LOX oxidation of ω-3 fatty acids and AA in a number of animal models including the mouse and rabbit [67] Further, work in cell lines confirms a role for these eicosanoids in a number of distant tissue beds as well. For example, mouse pre-adipocytes 3T3-L1 cells pre-treated with 12(S)-HETE and 12(S)-HpETE induce upregulation of proinflammatory cytokine genes, such as tumor necrosis factor-alpha (TNF-α), interleukin 6(IL-6), IL-12p40, and monocyte chemoattractant protein (MCP-1) [35]. In the human epidermis, 12(R)-HETE, which is sterochemically different from platelet-derived 12(S)-HETE, have been reported to increase DNA synthesis and plays a significant role in psoriasis [68, 69].

12-LOX Metabolite Regulation of Platelets

The role of the AA oxidized metabolite in platelets, 12-HETE, is not well understood. Furthermore, published work in this area of research is controversial as 12-HETE has been reported to be pro-thrombotic, anti-thrombotic, a well as inert towards platelet activity. One study indicated that 12-HETE had no effect on either basal or thrombin-induced $[Ca^{2+}]_i$ levels or aggregation [70]. Conversely, other reports showed a clear potentiation of thrombin-induced aggregation in platelets in the presence of 12-HETE [71]. 12-HpETE has also been shown to stimulate 12-LOX but inhibit COX-1 in lysed platelets[72] and one report indicated that 12(S)-HETE acts as an inhibitor of platelet and neutrophil PLA_2 activity [73]. Additionally, the eicosanoid, 15-HETE, which is primarily produced in leukocytes, has been reported to act as an inhibitor of 12-LOX [74]. Other eicosanoid products, such as 12-HPEPE and 12-HEPE originating from 12-LOX oxidation of EPA, are thought to elicit an inhibitory effect on platelet aggregation [54, 55]. In addition to their effects on aggregation, 12-HPEPE and 12-HEPE have been shown to attenuate serotonin (5-HT) release mediated by AA and collagen in a dose dependent manner [75]. The level of

fatty acid substrate available to 12-LOX may also play a role in its physiological regulation of platelet function. On the one hand, incorporation of DHA into one's diet lowers lipid peroxidation, which leads to attenuation of platelet reactivity at 200 μM [76] by inhibiting TXA_2 induced aggregation [77], whereas higher DHA concentrations of 400 μM resulted in increased prostaglandins [76]and potentiation of platelet reactivity. In a clinical trial, an elderly group receiving 30 mg of DHA per day resulted in lower lipid peroxidation levels and increased levels of vitamin E [78]. Additionally, elderly patients receiving 150 mg DHA and 30 mg EPA resulted in lower oxidative stress in platelets [57]. Contrary to these observations, one group has reported an increase in plasma lipid peroxidases in healthy individuals receiving 2 g EPA and 1.3 g DHA daily [79]. This paradoxical phenomenon has also been observed for the 12-LOX metabolite, 12-HEPE.

12-LOX Metabolite Regulation of CVD

Patients with essential hypertension, a significant risk factor for vascular occlusion [80], have shown an increase in the basal level of 12(S)-HETE in the platelets and in urinary excretion of 12(S)-HETE compared to healthy subjects [81]. Further, the protein levels of 12-LOX in the cytosolic fraction from hypertensive patients was much higher than in normotensive subjects. Thrombin-mediated 12(S)-HETE generation however, did not differ between the groups implying a potential role for genetic variation in the levels of 12-HETE formed [81]. SNP analysis has uncovered an association in the coding polymorphism of the 12-LOX gene with essential hypertension and urinary production of 12(S)-HETE [82].

Age is known to be directly correlated to an increased risk for cardiovascular disease [83, 84]. Similarly, platelet reactivity is also increased in older patients due, in part, to increasing levels of AA and TxA formation [85-87] Although 12-LOX product formation has not been studied in young versus older subjects, it is likely that 12-HETE is also increased in this segment of the population. Additionally, diabetics have been shown to exhibit an increase in PLA_2 activity linked to an increase in TxA_2 formation [88]. 12-LOX has been reported to have a link to platelet function in diabetes as well. 12-HETE levels in platelets from a group of Japanese patients with type 2 diabetes were observed to be decreased compared with healthy subjects [89], whereas noninsulin dependent patients in US showed an increase in 12-HETE formation in the urine samples compared to healthy subjects [90]. These controversial observations, although perplexing, support a link between the levels of 12-LOX activity in the platelet and diabetes.

12-LOX INHIBITORS

The studies described above show that regulating the amount of essential fatty acids and their metabolites *via* 12-LOX is essential in understanding both the pathophysiological processes of the platelets and CVD. Various groups have screened for potential natural and small molecule drugs targeting 12-LOX, however, many of these screens have failed due to problems with efficacy, off-target effects, and adverse events, both in animals and human platelets (Table 2). One of the earliest drugs tested on arachidonate 12-LOX was an acetylenic acid, 4,6-10-13-eicosatetrayonic acid (4,7,10,13-ETYA) [113]. This approach however, also targeted human peripheral neutrophil 5-LOX with an ID_{50} of 2-3 uM and other lipoxygenases from different sources and was therefore not developed further. Esculetin, also known as curcumin, was shown to inhibit 12-HETE production in both human and rat platelets [119], but did not inhibit formations of TxB2 and HHT [146]. Besides curcumin, baicalein (5,6,7-tihydroxyflavone), a compound extracted from *Radix Scutellariae* roots [96], was first reported to selectively inhibit 12-LOX in human platelets in the 1980s [147] without affecting cyclooxygenase activity [148]. In addition, platelet activation and ATP secretion stimulated by *Chlyamydia pnemoniae* was markedly reduced by this inhibitor [92]. More recent data suggests that baicalein inhibits $cPLA_2$ in human

platelets and that some of its effects may be due to a lower level of AA formation following initial platelet activation. Baicalein has also been reported to be an inhibitor of CYP2C9, an enzyme involved in drug metabolism [149] as well as other human LOs and COXs [95]. In addition to its' off target effects, baicalein in rats showed that the amount of 12-HETE produced in the presence of the inhibitor and thrombin stimulation did not correlate with the potentiation of contractile responses in the artery [150].

Other potential inhibitors which have shown little efficacy toward 12-LOX include 1) Dicranin (acetylenic fatty acid: 9,12,15-octadecatrien-6-ynoic aic), extracted from Dicranum Scoparium, which weakly inhibited COX-1, but resulted in an increase in 12-HETE [151], 2) Knipholone, which is isolated from the roots of *Kniphofa foliosa* and was shown to inhibit leukotriene synthesis, but only weakly inhibit 12-HETE production [152], and 3) OPC-29030 which inhibits thrombin-mediated 12(S)-HETE production [153]. Additionally, Hinokitiol, extracted from Japanese wood, was shown to be a selective 12-LOX inhibitor. Unfortunately, Hinokitiol has also been reported to be cytotoxic and terato-geneic on living tissues [129, 154]. Recently, there has been an increased interest in developing a highly selective small molecule inhibitor targeting 12-LOX. These compounds structurally exhibit greater selectivity than the previous natural inhibitors described above due to their selectivity in distinguishing and LO paralogs in species specific tissues/cells [145, 155]. These small molecule inhibitors may possibly reduce off-target effects in the system due to their greater selectivity and aid in clarifying the role of 12-LOX in the pathophysiology of thrombosis in the human.

CONCLUSIONS

Cardiovascular disease remains the leading cause of death in the world and is a growing problem both globally as well as within the United States. Research spanning over three decades has convincingly established a central role for platelet activation in the pathophysiology of cardiovascular disorders and acute coronary syndrome. Although current pharmacological therapy for treatment of diseases caused by blood clots, such as heart disease and stroke, has greatly improved the morbidity profile of patients with CVD, new approaches are warranted which alone, or in conjunction with currently approved pharmacological interventions such as aspirin or clopidogrel, will further decrease morbidity and mortality due to unwanted clot formation. Although targeting of enzymes such as COX-1 or surface receptors including $P2Y_{12}$, PAR1, and integrin receptor αIIbβ3, has been extremely useful in decreasing morbidity due to MI, these therapies have failed to significantly shift the incidence of mortality in these patients [156, 157]. This may be due, in part, to the fact that these anti-platelet drugs do not fully attenuate platelet activation, can have delayed onset and long durations of action, and may result in significant morbidity them selves due to bleeding complications [156, 157]. New therapeutic approaches targeting the level of platelet activation necessary to inhibit vessel occlusion and stroke without significantly increasing bleeding are needed. To address this problem, inhibiting platelet activation of a secondary pathway may allow for further inhibition of clot growth and stability without significantly altering the bleeding profile following vascular insult as is observed with inhibition of secondary pathways including COX-1 and $P2Y_{12}$. COX-1 inhibition for example, although only blocking formation of the weak agonist, TxA_2, has proven to be one of the most prescribed pharmacological approaches in anti-platelet therapy. 12-LOX may be a viable future target for anti-platelet therapy. Studies have shown that a link exists between the levels of 12-LOX and cardiovascular risks such as type 2 diabetes and hypertension. Furthermore, 12-LOX metabolites such as 12-HETE, have been shown to potentiate platelet activation, thrombin generation, and calcium mobilization. Recent work using small molecule inhibitors now supports a pro-thrombotic role for 12-LOX in the human platelet. Thus, targeting this enzyme in concert with inhibition of targets such as

COX-1 or $P2Y_{12}$ may allow for attenuation of the platelet clotting cycle without a significant increased risk of bleeding. The next phase of 12-LOX investigations should focus on whether inhibiting 12-LOX in both *in vitro* as well as *in vivo* animal model systems can either inhibit platelet activation to a greater extent compared to aspirin or clopidogrel, or determine if inhibition of 12-LOX works synergistically with the already established pharmacological approaches in order to potentially shift the threshold for platelet activation further to the right on an agonist dose-response curve.

Although 12-LOX was identified in the early 1970s by Hamberg and Samuelsson [158], identifying the regulatory role of 12-LOX and its metabolites in platelet function has been difficult, in no small part due to the poor selectivity of naturally occurring lipoxygenase inhibitors (see Table 2). Recently however, several research groups have revisited this enzyme and are developing a number of natural and synthetic molecule approaches in order to identify highly selective inhibitors against platelet-type 12-lipoxygenase in the human. The first generation of these inhibitors is now being tested in human platelets and early results support the potential targeting of this enzyme for future use as an anti-platelet therapy. Prior to establishment of 12-LOX as a viable target, suitable animal models will need to be identified in order to determine not only the effectiveness and safety of 12-LOX inhibitors, but to also identify the role of 12-LOX in hemostasis *in vivo*. Platelet-type 12-LOX is not the only potential target in development, however, its relatively selective expression in megakaryocytes and platelets, and its pro-thrombotic activity in human platelets supports further development of this target for anti-platelet therapeutics.

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ABBREVIATIONS

CVD	Cardiovascular diseases
12-LOX	Human 12-lipoxygenase
5-HT	Serotonin
AA	Arachidonic acid
ALA	α-linolenic acid
COX	Cyclooxygenase
DHA	Docosahexaenoic acid
DHETE	Dihydroxyeicosatetraenoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
ETYA	Eicosatetrayonic acid
GPCR	G protein-coupled receptor
HDHA	Hydroxydocosahexaenoic acid
HDOHE	Hydroxyl docosahexaenoic acid
HEPE	Hydroxyeicosapentanoic acid
HETE	Hydroxy-5,8,10,14-eicosatetraenoic acid

HHTE	Hydroxy-5,8,10-14-heptadecatetraenoic acid
HPETE	Hydroxy-5,8,10,14-eicosatetraenoic acid
IL	Interleukin
LA	Cis-linoleic acid
LO	Lipoxygenase
LT	Leukotriene
LX	Lipoxin
MCP-1	Monocyte chemoattractant protein
NSAIDS	Nonsteroidal anti-inflammatory drugs
PAR	Protease-activated receptor
PG	Prostaglandin
PLA	Phospholipase
PUFA	Polyunsaturated fatty acid
SNP	Single nucleotide polymorphism
TNF-A	Tumor necrosis factor-alpha
TX	Thromboxane

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Table 1

12-LOX Expression and Function in Different Species

Species	Cell Type	Function
Human	Adrenal glomerulosa cells	LO activation participates in aldosterone's stimulatory effects of angiotensin II (ANG) [37]
	Epithelial lens cells (HLECs)	Growth in response to EGF and insulin [38]
	platelet	12-HETE and other metabolites formation [26, 39]; platelet activation [2, 40]
Mouse	Pancreatic islets and adipose tissue	Cytokine production in adipocytes and macrophages shown to impair insulin signaling such as tumor necrosis factor-alpha (TNF-α), interleukin 6(IL-6), IL-12p40, and monocyte chemoattractant protein (MCP-1) [35]
	Melanoma cells	Cell proliferation [39, 41]
	Platelets	12-HETE and other metabolites formation shown to modulate platelet reactivity [42]
Porcine	Anterior pituitary	Possible gonadotroph regulation [43]
Rabbit	Corneal epithelial cells	Regulation of epithelial cell proliferation and the rate of corneal re-epithelialization following an injury [44]
Rat	Mesangial cells in renal	Indirectly involved in the expression of P-cadherins [45-47]
	Endothelium	Vasconstriction [48]
Canine	Gingival tissue, hepatocytes [49], Kupffer cells [50] and platelets [51]	Involved in neutrophil activity [50] and vasoconstriction and thrombosis [51]

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Table 2

12-Lipoxygenase Inhibitors and Targets

Inhibitor	Origin	Target(s)
CDC		12-LOX in human platelets; 5-LO in rats [91-93], 5-LO in human polymorphonuclear leukocytes and monocytes [93, 94]
Baicalein	Scutellariae roots	human reticulocyte 15-LOX and 12-LOX in platelets [27, 95, 96] , prolyl-4 hydroxylases (PHDs) in human hepatoma cells and mouse fibroblasts 3T3-L1 [97]; Raf-1 mediated phosphorylation of MEK-1 in rat glioma cells [98]; phosphatidylinositol 3-kinase (PI3K) inhibitor in human cancer cell lines e.g. colorectal, lung, gastric, ovarian, renal, melanoma, breast, glioma, prostate [99]
(NDGA)	Creosote bush	5-LOX, 12-LOX prostaglandin [100], inhibits HER2 and IGF-1 receptor tyrosine kinases in human breast cancer cells, [101, 102]; neutrophil phospholipase A2 [103]
BW755C	Phenidone analogue	dual inhibitor 12-LOX and COX-1 in platelets [104]; 5-LOX in leukocytes [105]
AA-861		5-LO in human and guinea pig peritoneal leukocytes [106]; 12-LO in mouse epidermis [107, 108]; tyrosine phosphatase of the receptor tyrosine kinase signaling pathway in mouse embryonic fibroblasts [109]
Timegadine		COX and LO in rabbit, rat, bovine, horse platelets [110-112] [110]
ETYA		12-LOX [113]; COX-1 [114-116], cytochrome P-450 ensymes in rat thyroid FRTLA-5 cells [117]; neutrophil phospholipase A2 [103]; Δ-6 desaturase in mouse hepatic cells [118]
Esculetin		12-LOX in human platelets [61, 119]; 5-LO [120],
BHPP	Hydroxamic acids	12/15-LO in rats and mice [121]
Panaxynol	a polyacetylene compound from Ginseng radix, Fang-Feng and Panx ginseng	COX, leukocyte 12-LO and platelet 12-LO in humans and porcine. 15-LO in rabbit [122]; cholesterol acyltransferase in rat liver [123]; 15-hydroxyprostaglandin dehydro-genase (PGDH) in rabbit gastric mucosa [124]
Falcarindiol	Panaxynol analog	leukocyte 12-LO and platelet 12-LO in human, and 15-LO [122]in rabbit reticulocyte; GABA degradative enzymes GABA transaminase (GABA-T) [125]
BW A4C	acetohydroaxmic acid	12- and 15-LO in bovine leukocyte, and 15-LO human leukocyte [126, 127]; secretory type II phospholipase A2 (sPLA2-II) in guinea-pig alveolar macrophages [128]
Hinokitiol	Derived from tropolone, a constituent of the wood *Chymacyparis taiwanesis*	12-LOX in human platelet [129, 130]; HIF-specific prolyl-4-hydroxylases (PHDs) [131] in human HepG2 hepatoma and HeLa cervical epithelial cells; metalloprotease in injects [132]
KY11449	Derived from *Streptoverticillium hadanonense*	Bovine 12-LO, rat basophilic leukemia cells (RBL-1) 12-LO [133]
Gossypol	Polyphenolic derived compound from cotton plant	5-LO and 12-LO [134] in human neutrophils and platelets, and rat basophilic leukemia cells (RBL-1) [135]; 11β-hydroxysteroid dehydrogenase (11β-HSD) activity in human and rat kidney [136]; Bcl-2 in human ovarian cancer cell lines OV433 and TOV112D [137]
Catechins	Green tea leaves	soybean lipoxygenases and 12-LO in rabbit and human [130]; tumor-associated NOX (tNOX) in human mammary adenocarcinoma cells (BT-20) and HeLa [138]

Inhibitor	Origin	Target(s)
Quercetin		LOs in rat mast cells, 12-LOX, and 5-LO in RBL-1[139]; neutrophil phospholipase A2 [103]; P-glycoprotein-mediated efflux transport and CYP3A4 enzyme [140], human glycolate oxidase [141], sPLA2 in human platelets [142]
MK 866		12-LOX in human platelets [127] , 5-LO in rats [143, 144]
12-LOX small molecule inhibitors	Small molecule screen	selectively targets 12-LOX in human platelets [145]