The 5 lipoxygenase system in the vasculature: Emerging role in health and disease

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Abstract

Activation of the 5 lipoxygenase (5LO) system within the vascular bed requires the presence of several cell types with distinct transcellular cross-talk mechanisms, resulting in the generation of 5LO produced metabolites and increased expression of receptors for these metabolites in vascular cells. The key products in this system, the leukotriens LTB4, LTC4 and LTD4, are potent mediators of vascular inflammation initiated by white blood cells and sustained or propagated thereafter through amplified metabolite generation and direct effects in endothelial and vascular smooth muscle cells. Leukotrienes act to enhance cell permeability and increase oxidative stress, vascular smooth muscle cell migration and arterial tone. 5LO activation is highly regulated, and is apparently both model/species-specific and region-specific. 5LO activation is also linked to plaque progression, plaque stability, activation of matrix metalloproteinases, propensity to coronary and cerebrovascular events and the evolution of aortic aneurysms. Genetic variants in the 5LO activating protein are strongly linked to increased cardiovascular risk and may serve as useful markers for future therapy targeting down regulation of 5LO expression and activity as a means to combat cardiovascular disease.

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1. Introduction

Lipoxygenase products are generated by nearly all cell types comprising the arterial wall per se, as well as by cells interacting with the vascular lining, i.e. polymorphonuclear leukocytes, monocytes, macrophages, lymphocytes and platelets. Following recent observations that propensity to cardiovascular events is linked to specific polymorphisms of the 5LO gene in a population-dependent manner, there has been a surge of interest in the role of 5LO in vascular disease. However, current understanding of the mechanisms underlying this association is limited. More than any other type of lipoxygenase enzyme, the vascular effects of 5LO system appear to depend on the interaction among several cell types comprising the arterial milieu. Although the physiologically important 5LO produced metabolites are all products of arachidonic metabolism, they are rather diverse in terms of origin, biosynthetic sequence and biological effects. Further, 5LO produced metabolites appear to exert differential effects in health and disease. While they are presently best known as mediators or instigators of local inflammatory vascular responses, their role in other vascular functions is the focus of current intense investigation.

2. Biosynthesis and regulation of 5LO produced eicosanoids

5LO is the first enzyme in a sequence of biosynthetic events culminating in the generation of multiple 5LO produced eicosanoids which are involved predominantly in local inflammation, vascular smooth muscle cell migration and modulation of vascular tone. As would be expected for a system that generates proinflammatory compounds, the 5LO pathway is abundantly expressed in macrophages, foam cells and inflammatory cells (dendritic cells, mast cells, and neutrophilic granulocytes) in atherosclerotic lesions of the aorta, coronary and carotid arteries. Further, the number of 5LO expressing cells markedly increased in advanced lesions (Spanbroek et al., 2003; Fiorucci et al., 2003). Many components of this system are detectable, however, only upon activation or in response to pathological
conditions, under which they tend to sustain and amplify the activating events. Therefore, SLO is tightly regulated by an array of signaling elements. First, it must be activated by a related peptide known as 5 LO Activating Protein (FLAP) which has three transmembrane-spanning regions and two hydrophilic loops. FLAP may function as a membrane anchor for SLO or as a membrane-independent activator of SLO, perhaps as a substrate transfer protein, which binds arachidonic acid or other fatty acid substrates for SLO activity. SLO resides in the cytosol in resting cells, but associates with the plasma membranes or nuclear membrane once the cell is activated (Radmark, 2000). Second, both the activation and the translocation of SLO to the nuclear membrane are calcium-dependent (Hammarberg et al., 2000).

Third, 5LO activity can be stimulated by ATP, and, to a lesser extent, also by ADP, AMP, cAMP, CTP, and UTP. Fourth, 5LO activity through phosphorylation at Ser271 and Ser663 by p38 MAP kinase (Werz et al., 2000, 2002a,b). The former can be activated by p38 MAP kinase (Werz et al., 2000, 2002a,b). Because both ERKs and p38 MAPks can, in turn, be activated by several proinflammatory cytokines, chemotactic factors, phorbol esters and Ca2+ mobilizing agents, cell stress induced by osmotic shock, UV light and heat shock, the potential for sustained SLO activation resulting in the formation of further local proinflammatory eicosanoids is obvious. Fifth, inhibition of SLO activation and translocation can be attained by cAMP-dependent PKA-induced phosphorylation of SLO at Ser523. Adenosine, prostaglandin E2 and beta adrenergic agonists are some of the extracellular agents capable of tuning down SLO activity through this mechanism (Phare et al., 2004).

The number of circulating or paracrine hormones known to regulate SLO expression is presently limited. Both 1, 25-dihydroxyvitamin D3 and transforming growth factor-beta (TGF beta) upregulate cellular 5 lipoxygenase activity in HL-60 cells through a process which also involves cell differentiation and maturation (Werz and Steinhiber, 1996). Several cytokines, such as granulocyte-monocyte colony-stimulating factor (GM-CSF) and TNF-alpha, potentiate the effect of TGF-beta. Additionally, GM-CSF and IL-3 can also directly stimulate expression of SLO (Radmark, 2000). In contrast, IL-4 appears to selectively down-regulate SLO but not FLAP in differentiating dendritic cells (Spanbroek et al., 2001). Notably, IL-4 as well as IL-13 induced down regulation of SLO is associated with induction of ISLO (type I) expression under these conditions.

The major biosynthetic events in the generation of SLO produced metabolites are illustrated in Fig. 1 (Martel-Pelletier et al., 2003). 5LO first introduces active oxygen to carbon 5 of arachidonic acid, resulting in the formation of 5HETE. This unstable derivative is either reduced to 5HETE or is converted to leukotriene (LT) A4. LT4A can serve either as an intracellular intermediate in the synthesis of LTs B4 and C4, or may be released extracellularly by activated leukocytes and subsequently be taken up by adjacent cells devoid of SLO activity but expressing LTA4-hydrolase and/or LTC4-synthase. Both enzymes are much more widely distributed than SLO. This process, termed “transcellular” biosynthesis, appears to facilitate amplification of LT production and LT-dependent inflammatory responses in the vasculature.

Thus, although endothelial cells are either entirely incapable of converting arachidonic acid into LTs, or can produce only very minimal amounts of LTs, as they are apparently practically devoid of (or possess only trace) SLO activity, these cells as well as VSMC can form LTB4 and LTC4 from LTA4 since they apparently express enzymes which act downstream in the synthesis of LTs: LTA4-hydrolase and LTC4-synthase. In this scenario, excessive adhesion of leukocytes to the vascular lining, which is induced under conditions predisposing to atherosclerosis such as hyperlipidemia, hypertension and locally turbulent blood flow or with actual leukocyte infiltration of the vascular wall in response to local injury, LTA4 thus generated, is likely to play an active role in transcellular biosynthesis of downstream, more vaso-active LTs, since the closer physical leukocyte/vascular cell interaction improves the transcellular transfer of the unstable LTA4 (Vila, 2003). However, there may be some negative feedback in this system in endothelial cells as exemplified by the finding that LTC4 inhibits the microsomal glutathione S-transferase type 2, the main enzyme responsible for its generation in these cells (Sjostrom et al., 2001).

Regardless of whether Leukotriene A4 was formed intracellularly or delivered extracellularly, it can be channeled by LTA4 hydrolase to form LTB4. Of note is the finding that LTA4 hydrolase-deficient mice display decreased capacity to recruit leukocytes in the vascular wall (Byrum et al., 1999). Alternatively, LTA4 can be conjugated with glutathione into one of the cysteinyl-leukotrienes termed LTC4, LTD4 or LTE4, shown in Fig. 1. The synthesis of LTC4 is catalyzed intracellularly by LTC4 synthase, whereas LTD4 and LTE4 can be generated from LTC4 extracellularly. LTB4 is generated mainly in neutrophils and macrophages.

3. Receptors for leukotrienes and effects of SLO produced metabolites

Once released, LTB4 increases chemotaxis for neutrophils; enhances neutrophil adhesion to endothelial cells; interferes with the anti-adhesive effect of aspirin; and augments vascular permeability. Leukotrienes B4 (LTB4) and the cysteinyl (cys) derivatives of leukotrienes, LTC4, LTD4, and LTE4 display strong proinflammatory activities in cardiovascular tissues. LTB4 also increases inflammatory responses indirectly, by stimulating the
formation of other proinflammatory mediators, i.e. IL-1, IL-2, INF-γ by T lymphocytes (Martel-Pellestor et al., 2003; Rocha et al., 2003). LTC4 was shown to upregulate the receptor for cysteinyl-leukotriene (cysLT1-R) action (see below; Sjostrom et al., 2001).

Currently, there are four presumed LT-activated, seven transmembrane domain, G protein-coupled receptors for leukotrienes, of which two apparently mediate LTB4 actions and are termed BLT-Rs (BLT1, BLT2). The other two receptors bind LTD4 and LTC4, and are referred to as cysLT1-R and cysLT2-R. CysLT1-R is expressed mainly in macrophages and blood leukocytes (Lotzer et al., 2003) and can be blocked by a group of classical CysLT1 antagonists including MK571, ICI198615 and poblukast (Norel and Brink, 2004). CysLT2-R is expressed in endothelial cells, can be antagonized by the non-selective CysLT blocker BAY94-8077 and is strongly upregulated by interleukin-4. Recent evidence suggests that both cysLT1-R and cysLT2-R receptors are expressed in rat VSMC and that the cysLT1-R is the dominant form in these cells (Mazzetti et al., 2003). A third CysLT receptor can be activated only by LTC4 and LTD4. In light of the increased expression of SLO in atherosclerotic lesions, it has been postulated that leukocytes, endothelial cells and T-cell might interact in self as well as mutually-enhancing inflammatory cross-talk. LT's originat- ing in macrophages or polymorphonuclear cells might acti- vate cysLT1-Rs in an autocrine or paracrine manner (Fig. 2). Leukotriens from these cells may also activate cystLT2-Rs on endothelial cells and cysLT1-Rs on T-lymphocytes in a paracrine fashion (Lotzer et al., 2003).

Since VSMC and endothelial cells can also produce leukotriens, at least under conditions such as hypertension, dia- betes and NO deficiency (Stanke-Labesque et al., 2001, 2003; Hardy et al., 2003), they comprise a potential source of agonists for the activation of cysLT1-R expressing macrophages which already migrated into subendothelial space or the media of the arterial wall. In human atherosclerotic plaques both CysLT1 and CysLT2 receptors are expressed (Forucci et al., 2003).

As inflammatory promoters, cysteinyl-leukotriens may also exert direct vasoactive effects and were shown to induce either vasoconstriction or vasodilation, in a species and/or vascular bed dependent manner (Berkowitz et al., 1984; Labat et al., 1992; Allen et al., 1994, 1998; Lawson et al., 1989). In human saphenous veins LTC4 and LTD4 induce vasodilation at very low doses and elicit vasoconstriction at higher doses, whereas coronary arteries appear unresponsive to either LTC4 or LTD4 (Norel and Brink, 2004; Allen et al., 1998). In contrast, in human atherosclerotic coronary arteries, LTC4 and LTD4 elicit a dose-related constrictor effect (Allen et al., 1998). When administered systemically, though, LTD4 increases blood pressure in rats in a dose-related fashion (Zukovska-Grojec et al., 1985). LTC4, LTD4 and LTE4 also induce vasoconstriction in distal segments of pulmonary arteries and in the mesenteric bed (Berkowitz et al., 1984). Consistent with the possibility that cysteinyl-leukotriens directly affect the contractile state of VSMC is the observation in cultured rat artery smooth muscle cells that LTD4 and LTC4 dose-dependently increased intracellular calcium concentration, which could be attenuated by montelukast, a selective type 1 CysLis receptor antagonist (Mazzetti et al., 2003).

However, under well-defined experimental conditions it appears that at least some of the leukotriene-induced vaso- constriction is endothelium-dependent. In one study, selective type 1 CysLis receptor antagonists dose-dependently prevented acetylcholine-induced contraction elicited in an endothelium-dependent preparation exposed to indometacin and preconstricted with phenylephrine (Mazzetti et al., 2003). In some instances, endothelium dependent vasodilation may also depend on some SLO produced metabolites, as illustrated by the observation that substance P-induced endothelium-dependent relaxa- tion in monkey and dog coronary arteries can be inhibited by the 5 lipoxygenase inhibitor AA861 (Fujioke et al., 2002).

There is also cumulative evidence suggesting that abnor- mal vasculature, such as presumably exists in hypertension and diabetes, exhibits increased formation of and/or sensitivity to various leukotriens. Indeed, under stressful vascular condi- tions, excessive vascular leukotriens generation can be turned on by vasopressor hormones, a mechanism which is turned off when the offensive factor (e.g., correction of hyperglycemia in diabetes) is removed. Indeed, such increased production of leukotriens can also contribute to altered regulation of blood pres- sure. For example, in L-NAME hypertensive rats, SLO block- ade with MK-886 ameliorates hypertension. Aortic tissue from L-NAME hypertensive rats increase the release of cysteinyl- leukotriens in response to norepinephrine, and norepinephrine-induced contraction of aortic rings from such L-NAME-treated rats (but not from control rats) can be attenuated by pretreat- ment with a SLO inhibitor, a CysLT1 receptor antagonist or by a dual CysLT1/CysLT2 receptor antagonist (Stanke-Labesque et al., 2003). In normal rats, angiotensin II-induced vasocon- striction appears not to depend on the SLO system and is not associated with changes in leukotriene generation. However, in aortas from the mRen-2/27 transgenic rats, which overexpress renin and develop hypertension, angiotensin II induced a large increase in CysLT formation and either the SLO inhibitor AA861 or the CysLT1 receptor antagonist MK571 attenuated the contactile response to angiotensin II (Stanke-Labesque et al., 2002). Indeed, in a mouse model overexpressing both human angiotensinogen and human renin, thus leading to hypertension,
responses to angiotensin II only in arterial preparations from neutralization of the 5LO axis either by the selective 5 lipoxygenase inhibitor AA-861 or the cysteinyl-leukotriene receptor antagonist MK-571 significantly reduced the vasoconstrictor responses to angiotensin II only in arterial preparations from SHR (Stanke-Labesque et al., 2001; Shafiei et al., 2001). Finally, cysteinyl-leukotrienes also participate in angiotensin II-induced contraction in diabetic rats, presumably another example of impaired vascular function, but not in control rats or in diabetic rats treated with insulin, via receptors distinct from the classical CysLT1 and CysLT2 receptors (Hardy et al., 2001). Collectively these data indicate that abnormal vasculature overproduces leukotrienes, at least in response to angiotensin II, and that these products then contribute to angiotensin II-dependent arterial tone. It is noteworthy that both atherosclerosis, as emerges from human studies, and abnormal arterial functional status, such as seen in the hypertensive and diabetic animal models, are linked to SLO activation. Therefore the potential for cross-amplification of SLO when hypertension and atherosclerotic lesions coexist requires testing.

5HETE was shown to increase endothelial cell growth via activation of Jak/STAT and phosphatidylinositol 3-kinase/Akt signalling, leading to induction of expression of basic fibroblast growth factor 2 (Zeng et al., 2002), but is otherwise understudied as a vasoactive compound. A membrane binding site for 5HETE was identified in human neutrophils (PMN). Several properties of the presumed action of 5HETE in neutrophils, such as mobilization of Ca++ and alteration in the binding of GTP, gamma S to the membrane suggest that 5-HETE acts by a down-regulatable, G protein-linked mechanism distinct from LTΒ4 receptors (O’Flaherty and Rossi, 1993).

4. Oxidative stress and SLO

Angiotensin II increases oxidative stress, in part, via SLO activity. It is well-established that angiotensin II-induced reactive oxygen species (ROS) formation is mediated, in part, through NAD(P)H oxidase derived superoxide anions. A recent study in VSMC showed that angiotensin II also induces the generation of LTΒ4, which then activates NAD(P)H oxidase, leading to ROS. The entire process can be inhibited by SLO blockade (Luchtefeld et al., 2003). NO depletion, another mechanism by which oxidative stress can be enhanced, was shown to increase the production of cysteinyl-leukotrienes in leukocytes perfused through coronary arteries, which could then be diminished by l-arginine (Buccellati et al., 1997).

5. Atherosclerosis and the 5 LO pathway

Though a fairly recently appreciated player in atherosclerosis, the SLO pathway is currently the focus of increasing attention with respect to the evolution of atherosclerotic vascular disease. First, SLO was abundantly expressed in atherosclerotic lesions of apoE (−/−) and LDLR (−/−) deficient mice (Mehrabian et al., 2002) as well as in human atherosclerotic arteries (Spanbroek et al., 2003). Further, SLO expression apparently correlates with the severity of atherosclerotic lesion in man (Spanbroek et al., 2003). Observations from our laboratory provide further support for this concept as SLO mRNA expression in the apoE (−/−) mice was 3.5-fold higher than in their genetic control mice (Osher et al., Unpublished observations). Second, even the absence of just one of the two SLO alleles confers significant resistance to atherosclerosis in a classical atherosclerosis-prone model such as the LDL receptor deficient mouse (Mehrabian et al., 2002). However, this association may be model-dependent as in mice null for the gene encoding SLO as well as apoE, no significant effects on the extent of atherosclerosis was seen (Zhao et al., 2004). Third, bone marrow from SLO (+/−) mice transplanted into LDLR (−/−), induced a significant reduction in atherosclerosis, thus implicating macrophage wild type SLO as a culprit in the atherogenic process (Mehrabian et al., 2002).

Fourth, an antagonist of the SLO produced metabolite LTB4 reduced monocye adhesions and atherosclerotic surface area in apoE (−/−) as well as LDLR (−/−) mice (Aiello et al., 2002). There is evidence that at least some of this effect may be due to the chemoattract effect exerted by LTB4 on VSMC (Heller et al., 2005). Furthermore, BLT1 (the receptor for LTB4) – knock-out mice bred into the atherosclerosis-susceptible apoE (−/−) strain had much less plaque formation than apoE (−/−) mice. Not only was the number of VSMC and macrophages reduced in the atheromas of BLT1-deficient mice, but BLT1-KO VSMC showed reduced migration responses in vitro in response to LTΒ4. Of interest is also the finding that while quiescent VSMC had no BLT1 expression, treatment with either TNF-[alpha] or IFN-[gamma] elicited a >25-fold increase in the expression of this receptor.

There is very recent evidence that SLO system may be linked to weakening of large artery walls and formation of aneurysms. Detailed histological analysis shows that most of the SLO-expressing macrophages in apoE (−/−) mice are located in the adventitia, where not only SLO expression but also the expression of FLAP and CysLT1R and CysLT2R increased with the progression of atherosclerosis (Zhao et al., 2004). Breeding mice null for SLO into apoE (−/−) mice resulted in reduction in the formation of aortic aneurysms. Indeed, SLO activation may also be one potential mechanism linking smoking to the formation of abdominal aneurysms in human subjects. Recent evidence indicates that nicotine can induce SLO expression in colon cancer cells (Ye et al., 2004). Further new evidence also links human carotid plaque instability and symptoms of carotid stenosis to increased expression of plaque SLO and elevated plaque concentration of LTΒ4, in association with increased expression of matrix metalloproteinase-2 and -9 (Cipollone et al., 2005).

Several independent population studies have indicated a link between the SLO system and vascular disease. One report concluded that specific variant genotypes of the SLO gene are significantly associated with increased atherosclerotic burden in
human subjects as assessed by carotid intima-media thickness and increases plasma C-reactive protein (Dwyer et al., 2004). In another report, a four-marker single nucleotide polymorphism (SNP) haplotype in the locus spanning the gene encoding 5LO-activating protein (FLAP) termed HapA was associated with a two-fold greater risk of myocardial infarction and stroke in Iceland. Another haplotype in the same locus, termed HapB, was associated with myocardial infarction in a British cohort. Of critical functional importance was the association in this study between this at risk haplotype and increased production of leukotriene B4 by activated neutrophils from male subjects (Helgadottir et al., 2004). In yet another study in a Scottish population, HapA, but not HapB, was again associated with ischemic stroke (Helgadottir et al., 2005). In a central European population, several other sequence variants in the FLAP gene, including one SNP which was formerly included in the Icelandic at-risk haplotype, were associated with stroke (Lõhmussaar et al., 2005). Although the precise mechanism by which these variants confer risk of MI and stroke remains unclear, these associations lend strong circumstantial support to the importance of 5LO-dependent mechanisms in human cardiovascular events.

In conclusion, there is mounting information that the 5LO system may play a critical role in arterial disease. Genetic predisposition to atherosclerosis may be, in part, related to facilitated activation of this system with enhanced formation of one or more of its vaculoactive metabolites. Indeed, therapy targeted to attenuate the activity of this system is presently under clinical experimentation and appears to lower biomarkers that are associated with increased risk of MI events in a genotype-related manner (Hakonarson et al., 2005). Better understanding of the vascular effects of 5LO produced metabolites may offer new opportunities to combat cardio- and cerebro-vascular morbidity and mortality.

References


