Review Article

Role of the Cysteinyl Leukotrienes in the Pathogenesis and Progression of Cardiovascular Diseases

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Cysteinyl leukotrienes (CysLTs) are potent lipid inflammatory mediators synthesized from arachidonic acid, through the 5-lipoxygenase (5-LO) pathway. Owing to their properties, CysLTs play a crucial role in the pathogenesis of inflammation; therefore, CysLT modifiers as synthesis inhibitors or receptor antagonists, central in asthma management, may become a potential target for the treatment of other inflammatory diseases such as the cardiovascular disorders. 5-LO pathway activation and increased expression of its mediators and receptors are found in cardiovascular diseases. Moreover, the cardioprotective effects observed by using CysLT modifiers are promising and contribute to elucidate the link between CysLTs and cardiovascular disease. The aim of this review is to summarize the state of present research about the role of the CysLTs in the pathogenesis and progression of atherosclerosis and myocardial infarction.

1. Introduction

The leukotrienes (LTs) are lipid mediators belonging to a large family of molecules named eicosanoids—from the Greek word “eicosa” meaning 20—as they are generated from the arachidonic acid (AA), a carbon-20 polyunsaturated fatty acid, through the 5-lipoxygenase (5-LO) pathway [1, 2].

The synthesis of LTs begins with the cleavage of AA from the glycerol-phospholipids present into the cellular nuclear membrane. The 5-LO, with the aid of the accessory 5-LO-activating protein (FLAP), catalyzes the conversion of AA to 5-hydroperoxyeicosatetraenoic acid (5-HETE) and then to leukotriene A₄ (LTA₄) [3, 4], an unstable intermediate, which can be either metabolized by LTA₄ hydrolase to LTB₄, a potent chemoattractant, or conjugated to glutathione by LTC₄ synthase (LTC₄S) producing the cysteinyl LTs (CysLTs: LTC₄, LTD₄, and LTE₄) [5].

The LTs exert their actions through interaction with specific 7-transmembrane G-protein-coupled cell surface receptors, BLT1 and BLT2, representing the high and low-affinity receptor for LTB₄, respectively, and CysLT₁ receptor (CysLT₁R) and CysLT₂ receptor (CysLT₂R) activated by the

CysLTs [6, 7] plus a recently discovered LTE₄-specific receptor known as CysLT_ER that was identified in CysLT₁R/CysLT₂R double-deficient mice [8]. The CysLTs present a different order of affinity for CysLT₁R and CysLT₂R. In detail, the rank of affinity toward CysLT₁R is LTD₄ > LTC₄ > LTE₄ whereas for CysLT₂R is LTC₄ = LTD₄ >> LTE₄ [9, 10]. GPR17 and GPR99, recently identified, may also be additional receptors for LTD₄/LTC₄ [11] and LTE₄, respectively [12]; moreover, LTE₄ has been reported to upregulate COX-2 through the PPAR γ receptor in mast cells [13], as well as to bind the P2Y₁₂ receptors [14]. As better detailed below, the CysLTs are synthesized by different cells and released in their extracellular space in response to several stimuli.

The effects of CysLTs in the cardiovascular system are established and suggest the existence of a solid link between the 5-LO pathway and cardiovascular diseases (CVDs) (Figure 1).

This review will focus on current knowledge about the involvement of the CysLTs in atherosclerosis and myocardial infarction and on the effects mediated by the CysLT modifiers on the disease progression.

FIGURE 1: The 5-LO pathway: biosynthesis, signaling, and effect on cardiovascular system. 5-Lipoxygenase (5-LO), leukotriene (LT), cytosolic phospholipase A2 (cPLA₂), arachidonic acid (AA), 5-LO-activating protein (FLAP), multidrug resistance protein-1 (MRP1), endothelial cells (ECs), and smooth muscle cells (SMCs).

2. CysLT Actors in Cardiovascular System

Atherosclerosis and myocardial infarction are vascular pathologies characterized by inflammation. The eosinophils, basophils, mast cells, and macrophages, major effector cells of innate immunity, possess the integral membrane protein LTC₄S [15] and are competent in synthesizing CysLTs in response to biological and nonbiological stimuli [16, 17]. Intriguing, cells unable to produce LTA₄, such as vascular endothelial cells [18], platelets [19], but also mast cells [20], blood peripheral monocytes [21], human airway epithelial cells [22], alveolar macrophages [23], kidney-derived

endothelial cells [24], keratinocytes [25], and chondrocytes [26], can use LTA₄ generated from the surrounding cells (such as neutrophils) to produce LTC₄ and the other CysLTs but also LTB₄. This process, called transcellular biosynthesis, could generate high concentrations of CysLTs at the local level, affecting organ function [27].

The CysLT₁R and CysLT₂R present distinct tissue and cellular pattern expression only partially overlapping [28]. Regarding the cardiovascular system, the expression of the CysLT₁R is hardly detectable [9, 29, 30], while that of the CysLT₂R is strongly expressed throughout the human heart, including the ventricles, atrium, septum, apex, and Purkinje

fiber cells [10, 30–34]. Moreover, CysLT₁R is present on monocyte and macrophages [35] whereas CysLT₂R on myocytes and endothelial cells (ECs) [10, 30, 35]. In smooth muscle cells (SMCs), conflicting expression has been reported among species. Indeed, CysLT₂R but not CysLT₁R has been detected in human coronary artery SMCs [30], while rat aortic SMCs express greater amounts of CysLT₁R protein compared with CysLT₂R and the intracellular calcium increase, induced by LTD₄, was inhibited by both the CysLT₁R antagonist montelukast and the dual CysLT₁R/CysLT₂R antagonist BAYu9773 [36].

The interaction of CysLTs with their receptors and the degree of their activation modulate several effects that could be relevant for the development of CVDs (Figure 1). Indeed, CysLTs exert negative inotropic action on the myocardium and decrease coronary blood flows with no effect on heart rate [37–41]; moreover, they may mediate contraction through the CysLT receptors on the endothelium or SMCs, as well as relaxation, which is endothelium dependent [42]. Furthermore, CysLTs can also stimulate proliferation of arterial SMCs and promote P-selectin surface expression, von Willebrand factor secretion, and platelet-activating factor synthesis in cultured ECs [43–45].

3. CysLT Modifiers: Change in Focus

The pathophysiological role of LTs in several inflammatory conditions and, particularly, in asthma is well documented, and several molecules, named *LT modifiers*, able to interfere with the LT biosynthetic cascade or with the LT receptors, have been approved for the treatment of asthma [46].

However, asthma may not be a classical comorbidity of cardiovascular disease; LTs have been implicated as potential mediators of cardiovascular risk in other inflammatory diseases.

In studies of patients with chronic obstructive pulmonary disease (COPD), characterized by high level of CysLTs [47], the prevalence of ischemic heart disease is almost twofold higher compared with the general population [48]. Based on this evidence, short-time treatment with the FLAP inhibitor BAYx1005 (DG031) has been evaluated both in patients with COPD and in patients with a history of myocardial infarction [49, 50]. However, although both treatment protocols resulted in only modest inhibition of LTB₄ concentrations, the overall results suggested a tendency for decrease of inflammatory markers [49, 50]. Recently, Hoxha and colleagues try to delineate the potential role of montelukast, the most described leukotriene receptor antagonist, in the treatment of cardiovascular diseases. Results from animal model studies [51–54] and from recent clinical trials [55, 56] show that montelukast, beyond its traditional use, can serve to prevent cardiovascular disease in humans and inhibit the atherosclerosis development in in vivo animal models suggesting a potential cardiovascular protective role [57]. Despite some limitations, all these studies provide an initial suggestion of a potential beneficial effect of an anti-LT treatment in cardiovascular disease; thus, there is a need for conducting clinical trials to assess the future role of these mediators in the CVD treatment.

4. Atherosclerosis

Atherosclerosis is a chronic inflammatory fibroproliferative process associated with several pathophysiological reactions within the vascular wall [58–60], characterized by (1) subendothelial oxidation of low-density lipoproteins (LDL); (2) infiltration of monocytes and their conversion to macrophages and lipid-laden foam cells; (3) accumulation of mast cells and other inflammatory cells; and (4) proliferation of smooth muscle cells and secretion of fibrous elements contributing to the growth of occlusive plaques [60]. This pathological condition can lead to myocardial infarction, stroke, and peripheral occlusive vascular diseases [61].

In human atherosclerotic lesions, increased expression of the 5-LO pathway mediators and products, including 5-LO, FLAP, LTD₄ hydrolase, LTC₄S, LTB₄, CysLTs, and CysLT receptors, was detectable [62, 63], suggesting the 5-LO pathway as a potential target for atheroprotective therapy (Figure 2).

The 5-LO-positive cells dramatically increased in advanced atherosclerotic lesions with progression from early to late stage of atherogenesis [63], and its expression has been mostly localized to macrophages which represent one major source of 5-LO [64], suggesting a possible role of 5-LO and its products in promoting lesion development [65].

In particular, a number of histochemical studies [63–66] pointed out that 5-LO was mostly present in activated CD68⁺ macrophages [63, 64] and that their distribution in lesions/plaque/aneurysmal arteries was not uniform. Indeed, the 5LO-positive cells were often observed at sites most prone to rupture [67], such as in the shoulder region below the fibrous cap, in the adventitia of diseased human arteries [63], in areas of neoangiogenesis, in granulomas around aneurysmal arteries [66], and also in neutrophilic granulocytes, dendritic, foam and mast cells [63].

From the time when the concept of inflammation and atherosclerosis was raised, a number of inflammatory mediators have been explored as potential therapeutic targets in this disease [68] and, among these, leukotrienes also have been investigated [35].

Although there is a long tradition of treating asthma with anti-CysLTs [69] and asthma may not be a classical comorbidity of atherosclerosis, some interesting indications were obtained from a randomized controlled trial of placebo versus the CysLT₁R antagonist montelukast, which reported significantly lower levels of C-reactive protein in treated patients with severe asthma [55]. Although no follow-up of those patients was performed in terms of cardiovascular disease, the systemic anti-inflammatory effect of montelukast could provide an initial suggestion of a potential anti-CysLT beneficial effect in atherosclerosis [70]. In fact, periodontal disease that could be ascribed as one of the sources of chronic inflammation is associated with an increased risk of stroke [71], myocardial infarction [72], and the development of early atherosclerotic lesions in the carotid artery [73]. In a study, it was found that subjects with atherosclerotic plaques and increased carotid artery wall thickness had significantly elevated concentrations of CysLTs in their gingival crevicular fluid as compared with subjects without a visible plaque [74].

FIGURE 2: Involvement of the 5-LO pathway in the pathogenesis and progression of atherosclerosis and major effects of CysLT modifiers in humans and in experimental models.

In addition to the studies implicating CysLTs in comorbidities of atherosclerosis, genetic and pharmacological experimental studies suggest the existence of a potential link between the CysLT signaling cascade and the pathogenesis/progression of atherosclerosis as well as its serious consequences such as myocardial infarction, brain ischemia, aortic aneurysms, and intimal hyperplasia [35, 75].

It was reported [76] that the identification of a locus on murine chromosome 6 that confers almost total resistance to atherogenesis and 5-LO was among the chromosome 6 locus candidates tested. The results showed that, in a congenic strain containing the resistant chromosome 6 (CON6), the mRNA levels of 5-LO and similarly 5-LO protein were reduced about 5-fold compared with the background strain.

A significant reduction in aortic lesions (more than 26-fold) observed in 5-LO^{+/-}/LDLR^{-/-} mice compared to 5-LO^{+/+}/LDLR^{-/-} mice further provides evidence of the involvement of 5-LO in the development of atherosclerotic lesions [65]. Moreover, it was reported that CON6 mice expressed a considerably reduced amount of 5-LO also in bone marrow and peritoneal monocytes/macrophages and that transplantation of CON6 or 5-LO^{+/-} bone marrow to LDLR^{-/-} mice had a similar effect on atherosclerosis (2- to 3-fold decrease) suggesting that the level of 5-LO in macrophages is responsible, at least in part, for the progression of atherosclerosis [65].

In addition, it was found that 5-LO genomic sequences of CON6 mice presented 2 nucleotide exchanges in the coding conserved region, which resulted into 2 amino acid exchanges of Ile-645 to Val (I645V) and of Val-646 to Ile (V646I) compared to wild-type mice, and that these murine mutations conferred an impaired 5-LO and LTA4S activity when introduced into the human enzyme [77].

A recent study [78] investigated the relationship between atorvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, and the 5-LO pathway mediators in an atherosclerotic rabbit model. New Zealand white rabbits subjected to carotid balloon dilation injury and treated with atorvastatin showed markedly lowered serum lipids and LTD4 levels compared with the control group. Similarly, mRNA expression of FLAP and CysLT₁R was significantly inhibited by atorvastatin. Moreover, atorvastatin treatment stabilized carotid plaque and decreased vascular inflammation as demonstrated by a thickened elastic layer, less neointima hyperplasia, and macrophage proliferation. This study suggested that atorvastatin might stabilize carotid plaque by regulating the 5-LO pathway in atherosclerotic rabbits and delay the progression of atherosclerosis by exerting anti-inflammatory effects. In contrast, high-dose simvastatin treatment induced overexpression of FLAP in patients' muscle [79] and two explanations are possible for the conflicting results: they could be attributable to the dosage forms of statins or species differences.

In human, the Carotid Atherosclerosis Progression Study [80] examined whether polymorphisms in 8 genes related to the 5-LO pathway were associated with early atherosclerosis and remodeling as measured by IMT. The results showed that these genetic variants had little effect on early atherosclerosis and remodeling risk. However, the subjects enrolled in this study represent a community population with predominantly early atherosclerosis, and there were insufficient advanced plaque and stenosis to exclude associations with advanced atherosclerosis.

Previously, a randomly sampled cohort of healthy subjects identified two variants of 5-LO genotypes (lacking the common allele) that were accompanied by a significant increase in IMT and atherosclerotic plaques [81]. In this population, dietary arachidonic acid significantly enhanced

the apparent atherogenic genotype effects and it was blunted by increased dietary intake of marine $n-3$ fatty acids (which reduced the production of LTs) suggesting diet-gene interactions.

On the contrary, in apolipoprotein E-deficient (ApoE^{-/-}) mice with either genetic (5-LO^{-/-}) or pharmacological (L-739,010) inhibition of the 5-LO and subjected to atherosclerotic regimen with either an 8-week Paigen or 6-month Western diet, any difference in atherosclerotic lesion size was observed between the groups [82]. Moreover, the composition of advanced lesions did not indicate an effect on plaque stability as a result of 5-LO gene inactivation [82]. Another study on ApoE^{-/-}/5-LO^{-/-} mice on a normal or Western diet showed no difference in atherosclerotic lesions compared to the control mice [66].

Despite 5-LO having to show a role in predisposition to atherosclerosis, taken together, all these controversial results do not clarify its role in the progression of pathology.

More convincing evidences of the involvement of the 5-LO pathway in atherosclerosis have been obtained evaluating FLAP [83]. In ApoE^{-/-}/LDLR^{-/-} mice, the administration of two different FLAP inhibitors, MK-866 [84] and BAYx1005 [85], showed a reduction in atherogenesis. The beneficial effect of MK-866 was also confirmed in transgenic ApoE^{-/-} x CD4dnT β R^{II} mice, with a dominant-negative TGF β type II receptor (dnTGF β R^{II}) on CD4⁺ T cells, which displayed aggravated atherosclerosis. The treatment with MK-866 significantly reduced the aortic root lesion size and also inflammation, as CD3⁺ cells and IFN- γ mRNA levels [86].

This antiatherosclerotic effect was also reported for the CysLT₁R antagonist. A reduction of atherosclerotic lesions in the aortic root was observed in ApoE^{-/-}/LDLR^{-/-} mice treated with CysLT₁R antagonist montelukast, even if in a lesser extent than FLAP inhibitors. This could be probably explained by "upstream" action of FLAP inhibitors on LT cascade, blocking both LTB₄ and CysLT productions, while montelukast inhibits the cascade "downstream" by blocking only the effect of CysLTs and leaving LTB₄ untouched [87]. However, as elucidated below, several studies established the effects of CysLT₁R antagonists on atherosclerosis [54, 88–91].

The role of LTC₄S has been investigated in the Muscatine study [92] which demonstrated the associations between coronary artery calcium (CAC) and intima/media thickness (IMT) (indices strongly associated with the amount of coronary atherosclerotic plaque) [93, 94] and the (-444) A > C promoter polymorphism of LTC₄S in woman, but not in men.

A significant increase in LTC₄S and CysLT₁R gene expression was observed in a model of ApoE^{-/-} atherosclerotic heart disease subjected to hypoxic stress [54] compare to wild-type control mice. Moreover, LTC₄S gene expression and activity and CysLT₁R gene and protein expression were enhanced in ApoE^{-/-} mice after bouts of hypoxic stress. Administration of the CysLT₁R antagonist montelukast reduced myocardial hypoxic areas suggesting a possible role of the CysLT pathway in oxygen supply. Accordingly, mRNA expression levels of LTC₄S and CysLT₁ were increased in human chronic ischemic compared to the nonischemic

myocardium, suggesting similar mechanisms to those observed in mice [54].

The multidrug resistance protein-1 (MRP1) was suggested as a mediator of the effect of LTC₄ on atherosclerosis. This protein acts as a transporter to the extracellular compartment [95] for LTC₄ as well as glutathione, oxidized glutathione, and estrogen [96] and is abundantly expressed in vascular SMCs and in human [97] and in the murine myocardium as well [98]. Its relevance in human health and disease has been deeply investigated [99], and it continues to be of considerable preclinical and clinical interest.

An *in vitro* study showed a proatherogenic mechanism mediated by MRP1 and LTC₄: pharmacological inhibition of MRP1 and CysLT₁R by MK571 and montelukast, respectively, reduced angiotensin II-induced ROS release in vascular SMCs [88]. Moreover, the *in vivo* study on atherosclerosis-prone ApoE^{-/-} mice, fed a high-cholesterol diet and treated with MK571 or montelukast for 6 weeks, showed a significant improvement in endothelial function and reduction of atherosclerotic plaque generation. These data represent an indirect proof of the MRP1 and LTC₄ roles in the atherosclerotic processes, indicating them as potentially promising targets for atheroprotective therapy [88].

Within atherosclerotic lesions, CysLTs, which are produced by coronary arteries [100], can locally mediate vascular reactivity exerting their effects by an autocrine and paracrine signaling [101]. Indeed, in addition to their well-known bronchoconstrictor effect, CysLTs, acting on SMCs, are also potent vasoconstrictors as observed in the human lungs [7, 102].

The hemodynamic effects induced by the CysLTs were evaluated in a small study on 6 patients without significant stenosis on a coronary angiogram but in which cardiovascular risk factors and coronary atherosclerosis cannot be completely excluded. The coronary vascular resistance, systemic mean arterial blood pressure, and heart rate were evaluated during and after the intracoronary LTD₄ administration (3 nmol bolus): no changes in resistance were observed during administration, while an increase was observed at 10 and 15 min after administration. Moreover, systemic mean arterial blood pressure initially decreased while heart rate was increased, returning to baseline after 10 and 1 min postinjection, respectively, suggesting that small doses of CysLTs induce both an early and transient fall in mean arterial pressure and a late increase in small coronary arteriolar resistance [103].

The urine levels of CysLTs increased in patients during and after acute myocardial infarction, unstable angina attacks [104], and coronary artery diseases both before and after coronary artery bypass surgery [105]. CysLT receptor subtypes are expressed in diseased human arteries, and hyperreactivity of atherosclerotic coronary arteries in response to LTC₄ was found to be associated with the expression of CysLT receptors [89].

In vitro studies on nonatherosclerotic human coronary arteries showed the lack of CysLT-induced coronary vasoconstriction [89, 105], although CysLT₂R mRNA expression can be detected in coronary artery SMCs [30]. In contrast, LTD₄ and LTC₄ induced contraction in atherosclerotic

coronary arteries which is inhibited by the CysLT₁R antagonist ICI198615 [89, 106] suggesting increased sensitivity to CysLTs during atherogenesis, probably due to an increased in the number of the binding site for LTD4 and LTC4 in atherosclerotic vessels [89, 105].

Furthermore, threefold higher levels of CysLT₁R transcripts compared with CysLT₂R transcripts were observed in atherosclerotic lesions from human carotid arteries [90] and an increased CysLT₁R expression in the aorta was observed in atherosclerotic ApoE^{-/-} mice, compared with nonatherosclerotic mice [66].

A more recent study [107] showed colocalization of the CysLT₁R protein with markers for SMCs in human atherosclerotic lesions revealing also CysLT₁R predominant perinuclear localization compared with cytoplasmatic alpha-smooth muscle actin localization. This study also showed an upregulation of CysLT₁R induced by inflammatory conditions (LPS, L-6 and by prolonged exposure to IFN- γ). Taken together, all these observations suggest that a proinflammatory environment, such as atherosclerosis, may induce CysLT₁R expression within the SMCs in the vascular wall and a major role of the CysLT₁R in atherosclerosis compared to CysLT₂R was observed.

Similar findings have been reported in EC, which under resting conditions exhibit a dominant CysLT₂R, but in which a prolonged exposure to LPS or to proinflammatory cytokines upregulates CysLT₁R expression [108]. Recently, an *in vitro* study [91] showed that LTC4 and LTD4 induce robust calcium influx in human umbilical vein endothelial cells (HUVECs), which was significantly inhibited by both Rho kinase inhibitor (Y27632) and CysLT₂R antagonist (BayCysLT2), but not by CysLT₁R antagonist (MK571), suggesting that contraction of EC, induced by LTD4, was mediated only by CysLT₂R [91]. LTC4 and LTD4 also stimulated EC proliferation, which was completely blocked by a MEK inhibitor (PD98059) and inhibited by MK571, indicating the CysLT₁R role in EC proliferation. In the same study, CysLTs significantly increased the TNF α -induced expression of the adhesion molecule VCAM-1 and attachment of leukocytes to ECs. Notably, the recruitment of leukocytes was significantly attenuated by BayCysLT2 but not by MK571 [91].

Furthermore, LTC4 and LTD4 increased the expression of the adhesion molecule P-selectin in human ECs [45, 109]. This increase was not inhibited by CysLT₁R antagonists, suggesting a CysLT₂R-induced effect. In HUVECs, CysLT₂R activation may also induce other proinflammatory effects through increased transcriptional activity [110]. Indeed, the LTD4-induced upregulation of IL-8, CXCL-2, and COX-2 was not inhibited by CysLT₁R antagonist but seems to be sensitive to synergistic effects between CysLT2 and protease-activated (PAR-1) receptors. Taken together, these results suggest that CysLTs increase, in a CysLTR-dependent manner, EC proliferation and expression of inflammatory genes involved in the recruitment and adhesion of leukocytes, which play a critical role in the etiology of atherosclerosis.

In addition to ECs and SMCs, also, T lymphocytes are involved in atherosclerosis, and despite the fact that these cells might not express CysLT receptors, CysLTs could

potentially modulate adaptive immunological reactions by inducing the activation of antigen-presenting cells.

In a murine model of asthma, myeloid dendritic cells were shown to express CysLT₁R, and LTD4 stimulation increased the production of the immunomodulatory cytokine IL-10, which was inhibited by treatment with CysLT₁R antagonists [111].

It has been shown that interleukin IL-10 overexpression can inhibit fatty-streak formation in C57BL/6J mice fed an atherogenic diet containing chocolate [112, 113]. Furthermore, in LDLR^{-/-} mice, the overexpression of IL-10 by T cells induced a significant decrease in lesion size and necrotic core, inhibiting advanced atherosclerotic lesions [114]. Moreover, the accumulation of cholesterol and phospholipid oxidation products in the aorta was decreased by 50% to 80%, unrelated to plasma lipid or IL-10 levels [114]. In line, IL-10 deficiency in ApoE^{-/-} (IL-10^{-/-}/ApoE^{-/-}) mice increased atherosclerotic lesion size compared with ApoE^{-/-} control mice [115]. These studies indicated as the production of IL-10 induced by LTD4 could have a protective role in the atherosclerotic process and suggested that CysLT signaling may represent one possible regulator of immunomodulatory functions in atherosclerosis.

5. Myocardial Infarction

The possible involvement of LTs in the development of myocardial infarct damage has been of considerable interest within recent years. The genetic variants within the 5-LO pathway are associated with an increased risk of stroke and myocardial infarction (MI) [75]; moreover, the production of CysLTs increases in ischemia-reperfusion injury in both patients and animal models.

Because of their rapid metabolism and excretion, LTs are difficult to be measured accurately in blood [116, 117], although elevated plasma concentrations of these mediators have been reported after acute MI [104]. They influence, directly or indirectly, coronary vascular resistance, infarct size, pulmonary vascular resistance, bronchial tone, and renal vascular resistance; moreover, they are key regulators of inflammation and thus potential targets to influence healing after MI [5].

Development of MI injury is characterized by three phases: ischemic, reperfusion, and inflammatory. This last phase is characterized by increased expression of cell adhesion molecules, as well as leukocyte infiltration in a manner similar to those observed during inflammatory reaction [118]. In this process, the inflammatory cells, invading myocardial tissue after infarction, or their metabolic products, play a crucial role in the development of the damage and may participate in reperfusion injury [119]. The importance of leukocyte in cardiovascular disease has recently been reviewed [120], and several reports indicate a correlation between myocardial infarct size and the magnitude of leukocyte infiltration [121, 122]. Among a number of inflammatory mediators regulating leukocytes, LTs should be included. Indeed, LTs are necessary for the function and migration of leukocytes [91, 109]; moreover, LTs could play a role in the development of MI since they influence

fibroblasts [30], increase contractility and proliferation of smooth muscle cells [89, 106, 123], and are also important for vascular permeability [124].

In animal models, experimental myocardial infarction causes elevated LT production in the damaged tissue and evidence suggests that 5-LO products exert a detrimental role in tissue recovery. Several investigators have pharmacologically tested the effect of lipoxygenase inhibitors on ischemic injury [125]; however, since results are controversial, a number of transgenic mice were studied to overcome the limitation of unspecific responses by pharmacological agents.

Adamek and colleagues [126] determined the response to ischemia/reperfusion injury in mice with targeted disruption of 5-LO. The 5-LO-deficient mice exhibit an increased neutrophil infiltration and proinflammatory gene expression within the infarction area compared with wild-type mice. Nevertheless, authors report that, despite an important role of 5-LO in inflammatory responses, 5-LO seems to not play a major role in ischemia-reperfusion injury in the heart. These data compared with investigation made in other organs [127–129] hypothesize that 5-LO effects might be organ specific. However, although these results raise a doubt on to the role of LTs in myocardial ischemia, a number of evidence from other studies support their strong involvement.

Recently, in a large Danish cohort study [130, 131], the association between 20 preselected single-nucleotide polymorphisms (SNPs) and MI events has been evaluated, demonstrating that some common SNPs in the 5-lipoxygenase pathway were modestly associated with incident MI, suggesting a potential role for this pathway in the development of cardiovascular disease.

As reported for atherosclerosis, MRP1 seems to mediate, at least in part, the cardiac effects of LTC₄. A recent study has suggested an important role of MRP1 on intracellular redox homeostasis and myocardial performance [51]. In this study, the cardiac effects of CysLT₁R blocker montelukast and MRP1-inhibitor MK571 as well as MRP1 depletion were tested *in vitro* and *in vivo*. Results demonstrated that pharmacological blockade of CysLT₁R prevents LTC₄-induced ROS production and release in cultured cardiomyocytes and, additionally, that montelukast reduces oxidative stress and apoptosis in cardiomyocytes having a beneficial effect on myocardium remodeling and improves myocardial function after left ventricular injury in a mouse model of crio-induced MI. Moreover, the inhibition of LTC₄ transport, either in MRP1^{-/-} mice or in MK571-mediated mice, resulted, *in vivo*, in reduced oxidative stress and apoptosis and demonstrated beneficial effects on cardiac remodeling after injury.

On the contrary, the role of LTD₄ in MI is not clearly elucidated. LTD₄ is one of the leukocyte metabolites with high coronary constrictor potency, mainly released from macrophages [132, 133] but also produced by a variety of tissues, including coronary and pulmonary arteries [134]. In a model of coronary stenosis and myocardial ischemia, LTD₄ induced coronary constriction [135]; moreover, it was reported that its levels increased in infarcted rabbit hearts [136] and in urine of humans with acute cardiac ischemia [104]. LTD₄ acted also as potent coronary vasoconstrictor in the isolated rat heart model, and this effect was more potent in chronically infarcted

heart [137]. Intravenous administration of LTD₄ produced prominent cardiovascular alteration in rat and dog, characterized by a decrease in blood pressure and a reduction in aortic arterial blood flow and stroke volume. Nevertheless, the administration in rat and dog of LY203647, described as a potent and selective antagonist of responses to both LTD₄ and LTE₄, did not alter the magnitude of myocardial ischemia [138]. The limitation of the influence of the endogenously produced LTD₄ in the progression of cardiac damage was also confirmed in another study where an alternative specific antagonist L-660,711 had no effect on coronary blood flow and cardiac performance in rats following MI [137].

Aforementioned, the CysLTs exert their effects by binding to G-protein-coupled receptors CysLT₁R and CysLT₂R and novels GPR99 [12] and GPR17 [11].

To clarify which receptor was mediating the most of the cardiovascular CysLT effects, the consequences of the HAMI3379 and zafirlukast, CysLT₂R and CysLT₁R antagonists, respectively, were tested on LTC₄-treated, Langendorff-perfused, guinea pig hearts [139]. Results showed that HAMI3379 was an effective antagonist of the cardiac effects of LTC₄, while zafirlukast was found to be inactive in this experimental setting, suggesting that the cardiac CysLT effects are mainly mediated by CysLT₂R and these results were in good agreement to the high expression of the CysLT₂R in the heart and blood vessels. In another study [140], the treatment with BayCysLT₂, a potent CysLT₂R antagonist, attenuated increased infarction damage when administered either before ischemia or after reperfusion. This treatment prevented the increases in cell adhesion molecule gene expression and leukocyte infiltration into the myocardium, both hallmarks of the acute inflammatory response following MI. These findings indicate that CysLT₂R activation results in heightened facilitation of diapedesis, which enhances the magnitude of the inflammatory response leading to additional damage to the site of injury [140]. This mechanism was then confirmed by an *in vivo* study [124].

Using the CysLT₂R transgenic mice, overexpressing human CysLT₂R in vascular endothelium, as well as knockout mice, a role for the CysLT₂R in vascular permeability and myocardial ischemia/reperfusion injury has been shown [33, 34, 141]. In particular, the endothelial overexpression of CysLT₂R [141] increased cardiomyocyte apoptosis in the peri-infarct region and induced an exacerbation of damage after MI resulting, from signaling through this receptor, in an increase in CD45⁺ cell infiltration, intermyofibrillar erythrocyte accumulation, and fluid extravasation worsening inflammatory gene expression and increasing infarct size.

On the contrary, the overexpression of CysLT₂R also unaltered left ventricular function in uninjured myocardium [141].

The mechanism of action was partially explained in another study on the same model by demonstrating that CysLT₂R mediates inflammatory reactions in a vascular bed-specific manner by altering transendothelial vesicle transport-based vascular permeability [34]. A very recent paper indicated the existence of endothelial and nonendothelial CysLT₂R niches having separate roles in mediating inflammatory responses in which activation is required for

injury exacerbation [124]. Particularly, endothelial receptor activation results in increased vascular permeability and leukocyte slow rolling, facilitating leukocyte transmigration, whereas nonendothelial receptors, likely located on resident/circulating leukocytes, facilitate leukocyte recruitment to the site of injury and activation of endothelial receptor.

GPR17 is a P2Y-like receptor responding to both uracil nucleotides and LTD4/LTC4 whose presence characterizes various organs susceptible to ischemic damage such as brain, kidney, and heart [11]. Moreover, it can interact with other closely related receptors, since its ability to act as a negative regulator of the CysLT₁R [142, 143] was recently reported, as previously hypothesized by Maekawa and collaborators both *in vitro* and *in vivo* [8, 144, 145]. In normal mice, it was found expressed in cardiac-resident stromal cells [146] suggesting the same role observed in the central nervous system, where GPR17⁺ cells seem to have a role of a damage “sensor” able to activate healing program [147], whereas, following MI, GPR17 was found in resident and recruited CD45⁺ cells [146]. Interestingly, it was found that the treatment of the cardiac stromal cells with LTD4 exerted a potent chemotactic effect via GPR17 activation and that this effect can be reverted by cotreatment with montelukast, a GPR17 pharmacological antagonist [146]. These findings point to a specific GPR17 role in chemotactic guidance of stromal cells towards the ischemic sites and open to the hypothesis that the selective modulation of GPR17 signaling translates into beneficial treatments potentially reducing the extent of myocardial fibrosis and limiting the functional consequences of heart ischemia.

6. Summary

Cysteinyl leukotrienes are lipid mediators inducing pro-inflammatory signaling through the activation of specific receptors.

Exciting preclinical and clinical data indicate that the 5-LO pathway becomes activated in cardiovascular diseases and suggests an important role of CysLTs in atherosclerosis and in its ischemic complications such as myocardial infarction and stroke. Moreover, CysLT modifiers, generally safe and well tolerated, approved for the treatment of asthma, show significant cardioprotection in the experimental setting. To date, the information available give emphasis to CysLTs as potential targets in cardiovascular diseases and may provide the necessary background and justification to launch novel therapeutic programs. Nevertheless, further experimental and clinical studies are needed to determine the potential of therapeutic strategies targeting the 5-LO pathway in cardiovascular disease and the link existing between the human genetics and the 5-LO pathway in the inflammatory pathology of cardiovascular diseases.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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