Involvement of Leukotriene Pathway in the Pathogenesis of Ischemia-Reperfusion Injury and Septic and Non Septic Shock

Antonietta Rossi[1], Salvatore Cuzzocrea[1,2,*] and Lidia Sautebin[3,*]

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[1]*IRCCS Centro Neurolesi "Bonino-Pulejo", Via Provinciale Palermo C. Da Casazza, 98124 Messina, Italy;* [2]*Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, Via C. Valeria, 5-98125 Messina, Italy;* [3]*Department of Experimental Pharmacology, University of Naples Federico II, Via D. Montesano, 49-80131 Naples, Italy*

Abstract: The 5-lipoxygenase (5-LO) pathway is responsible for the production of leukotrienes (LTs), inflammatory lipid mediators which play a role in innate immunity. More recently, a pivotal role of LTs in ischemia-reperfusion and shock injury has been suggested. Infact, these pathological conditions are characterized by a severe neutrophil infiltration that gives rise to tissue injury and 5-LO metabolites control neutrophil recruitment in injured tissue by the modulation of adhesion molecule expression. The aim of this review is to analyze the results reported in the literature on the role of 5-LO pathway, with particular regard to LTs, in these pathological conditions. A better understanding of the mechanisms underlying the role of the 5-LO enzyme and/or its metabolites in the regulation of neutrophil trafficking, might open new perspectives in the therapy of organ dysfunction and/or injury associated with shock and ischemia-reperfusion injury.

Keywords: 5-lipoxygenase, adhesion molecules, ischemia-reperfusion injury, leukotrienes, neutrophil infiltration, septic shock, non-septic shock.

1. LEUKOTRIENES

1.1. Leukotriene Biosynthesis

Leukotrienes (LTs) ("leuko" from white blood cells and "trienes" three conjugated double bonds) are products of the 5-lipoxygenase (5-LO) pathway. The biosynthetic pathway of LTs initiates with the migration of 5-LO and cytosolic phospholipase A_2 ($cPLA_2$) to the nucleus, where $cPLA_2$ liberates arachidonic acid AA from phospholipids, which is then transferred by an integral membrane protein 5-lipoxygenase-activating protein (FLAP) to 5-LO. For full activity 5-LO requires cofactors (calcium and ATP) and interaction with other proteins besides FLAP, such as the coactosin-like protein (CLP), which stimulates 5-LO activity and colocalizes with the enzyme [1]. The production of LTs begins with the insertion by 5-LO of molecular O_2 at carbon-5 of AA to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE). Five-HPETE can be reduced to 5-hydroxyeicosatetraenoic acid (5-HETE), which can be in turn dehydrogenated to 5-oxo-ETE. Moreover, 5-LO catalyzes a second enzymatic step, the conversion of 5-HPETE to leukotriene A_4 (LTA_4), an unstable intermediate [2] that can be catalytically converted to leukotriene B_4 (LTB_4) by LTA_4 hydrolase [3] or can be conjugated with reduced glutathione by leukotriene C_4 (LTC_4) synthase (LTC_4S). LTB_4 and LTC_4 are exported from the cell by specific transporter proteins. The LTC_4 is subjected to extracellular cleavage of the glutamic acid (through a γ-glutamyl transpeptidase) and subsequently of the glycine moiety (through a dipeptidase), to provide respectively leukotriene D_4 (LTD_4) and E_4 (LTE_4) [4].

Interestingly, it has been demonstrated that there are allelic variants of the coding and promoter regions of the genes encoding for the enzymes involved in LT biosynthesis, such as 5-LO [5], FLAP [6], LTA_4 hydrolase [7] and LTC_4S [8].

The LT synthesis is regulated by different mechanisms involving: a) the amount of free arachidonate released by PLA_2 from cell-membrane phospholipids, [9, 10], b) the availability of small molecules (e.g. ATP), c) the level and the catalytic activity of each of the proteins involved in the 5-LO pathway, and, d) the 5-LO post-translational modifications [e.g. phosphorylation by p38 kinase-dependent mitogen-activated protein kinase (MAPK) [11], phosphorylation on Ser 663 by extracellular signal-regulated kinase (ERK)] [12]. Another variable that influences LT synthesis is the intracellular localization of 5-LO. In resting cells, 5-LO occurs as a soluble enzyme either in the cytosol or in the nucleus, depending on the cell type [12]. In neutrophils, cytoplasmic 5-LO associates with the endoplasmatic reticulum and with the outer nuclear membrane, whereas in dendritic cells or in alveolar macrophages has an intranuclear localization that seems to be correlated with a higher capacity for LT generation [13]. Cell stimulation by various agonists causes 5-LO translocation from soluble compartments to the nuclear membrane and the consequent LT generation. LTs are predominantly synthesized by inflammatory cells like polymorphonuclear leukocytes (PMNs), monocytes, macrophages, mast cells and dendritic cells [for review see 14]. Although non leukocyte cells (like endothelial cells) generally do not

*Address correspondence to these author at the Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, *Via* C. Valeria, 5-98125 Messina, Italy; Tel: ++39-0902213644; Fax: ++39-0902213300; E-mail: salvator@unime.it and Department of Experimental Pharmacology, University of Naples Federico II, Via D. Montesano, 49-80131 Naples, Italy; Tel: ++39-081678427; Fax++39-081678403;
E-mail: sautebin@unina.it

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have sufficient 5-LO and FLAP to synthesize appreciable amounts of LTs, such cells expressing only LTA_4-metabolizing enzymes can take up leukocyte-derived LTA_4 and metabolize it into bioactive LTs, a process that is termed "transcellular biosynthesis"[15].

1.2. Leukotriene Receptors and Biological Effects

LTs exert their actions through seven-transmembrane G protein coupled receptors consisting of 2 subclasses: receptors activated by LTB_4 (BLT receptors) and cysteinyl LT (cysLT) receptors, activated by the cysLTs (LTC_4, LTD_4 and LTE_4) [16].

These receptors, after binding to LTs, interact with G proteins, thereby eliciting increases in intracellular calcium and reductions in intracellular cyclic AMP [for review see 14]. These proximal signals activate downstream kinase cascades in ways that alter various cellular activities, ranging from motility to transcriptional activation.

The BLT receptors are denoted BLT_1 and BLT_2, based on their affinity for the agonist [17]. In particular, LTB_4 acting through BLT_1 can cause chemotaxis, degranulation, adhesion and an enhancement of the neutrophil survival. Although BLT_1 was long known to be a neutrophil chemoattractant receptor, recent studies identified BLT_1 expression in macrophages, smooth muscle cells, endothelial cells, activated T-cells, mast cells, eosinophils and basophils [for review see 14]. Functional expression of BLT_1 on both mature and immature dendritic cells has been recently demonstrated supporting a direct effect of LTB_4 in the control of adaptive immune responses [18]. The use of knockout (KO) mice for BLT_1, generated in several laboratories, have been fundamental in defining a critical role for this receptor in diverse inflammatory diseases, such as atherosclerosis [19], asthma, autoimmune uveitis and arthritis [for review see 20]. In contrast, the function and biological activities of BLT_2 are completely unknown, although this receptor is expressed on neutrophils, macrophages, T lymphocyte, mast cells, B lymphocyte, eosinophils, dendritic cells and hematopoietic progenitor cells [for review see 14]. Moreover, this receptor has been shown to be expressed widely in humans being with the highest expression in spleen and peripheral blood leukocytes [21]. However, varying results has been reported as regard to murine BLT_2 expression [22-24]. Recently, it has been reported that BLT_2 expression portends worse clinical parameters for ovarian cancer [25].

The receptors activated by the cysLTs are referred to as $CysLT_1$ and $CysLT_2$ [26]. $CysLT_1$, [27] mediates sustained bronchoconstriction, mucus secretion, and edema in the airways. Selective antagonists of $CysLT_1$, that are approved for the treatment of asthma, block the proasthmatic effects of $CysLT_1$ stimulation. Experiments in mice that are deficient in $CysLT_2$ [28] or that overexpress $CysLT_2$ in the lungs [29] indicate that $CysLT_2$ does not mediate bronchoconstriction but, rather, contributes to inflammation, vascular permeability and tissue fibrosis. There are no known specific antagonists of $CysLT_2$. Interestingly, certain reported actions of cysLTs are not readily explained by either $CysLT_1$ or $CysLT_2$, raising the possibility of the presence of $CysLT_1$–$CysLT_2$ heterodimers or additional receptors [30]. One candidate is G protein –coupled receptor 17 (GPR17), a dual-uracil nucleotide–cysLT receptor [31].

1.3. Leukotrienes and Diseases

LTs play an integral role in the pathophysiology of asthma. In fact, the role of LTs in this disease has been validated in clinical trials of anti-LT agents. In particular, the anti-LT therapy (zileuton, a 5-LO inhibitor [32], and montelukast or zafirlukast [33, 34], $CysLT_1$ antagonists) improve pulmonary function, decreases daytime and nocturnal bronchoconstriction episodes and increases quality of life in children and adults with asthma. Recently, it has been demonstrated that anti-LT agents exert beneficial effects on other diseases commonly associated with asthma (exercise induced asthma, rhinitis, chronic obstructive pulmonary disease, interstitial lung disease, chronic urticaria, atopic dermatitis, allergic fungal disease, nasal polyposis, and paranasal sinus disease) as well as on diseases not connected to asthma [35]. In fact, the overproduction of LTs has been also associated with atherosclerosis [36, 37], hyperlipidemia-dependent inflammation of the arterial wall [38], pulmonary hypertension [39], arthritis (including osteoarthritis and gout) [40], glomerulonephritis, interstitial cystitis and psoriasis [14]. In addition, increased 5-LO expression, and presumably increased LT synthesis, has been associated with several tumour types such as lung [41], pancreatic [42], bladder [43], breast [44], colon [45], multiforme glioblastoma [46], prostate [47], testicular [48] and esophageal cancer [49]. The role of LTs in carcinogenesis seems to be related to their action on fundamental cellular processes such as differentiation and proliferation through the transcription of various cytokines and growth factors [50]. Recently, we have demonstrated by the use of 5-LOKO mice, 5-LO inhibitor and $CysLT_1$ receptor antagonist, that LTs are important mediators of several pathological conditions characterized by an excessive neutrophil activation (pleurisy [51], acute pancreatitis [52], colitis [53, 54], spinal cord injury [55], septic and non septic shock [56, 57] and ischemia/reperfusion injury [58, 59]) by promoting neutrophil migration through an up-regulation of adhesion molecule expression.

2. INVOLVEMENT OF LEUKOTRIENES IN THE PATHOGENESIS OF ISCHEMIA-REPERFUSION INJURY

2.1. Ischemia-Reperfusion Injury

Ischemia and reperfusion (I/R) injury, that develops when blood flow is interrupted for a long period of time and then restarts, occurs in a wide range of situations, including trauma, vascular reflow after contraction, transluminal coronary angioplasty, thrombolysis treatment, organ transplantation and hypovolemic shock with resuscitation. When a tissue is subjected to ischemia, a sequence of chemical reactions is initiated that may ultimately lead to cellular dysfunction and necrosis. In fact, if the ischemia is severe enough, the rate of metabolism is diminished and the generation of high energy compounds subsequently declines (e.g. ATP). The reduced energy metabolism eventually leads to a slow but significant degree of tissue injury and necrosis. This degree of tissue injury is further enhanced and accelerated by reperfusion that leads to reoxygenation and to the formation

and activation of a variety of humoral mediators of injury and inflammation. Another key factor of reperfusion injury is PMN activation and infiltration in the ischemic area. Multiple studies identified consecutive stages of PMN activation and substances being involved in it [60]. It has become apparent that PMN infiltration is not, as once thought, a secondary phenomenon following ischemia. Rather, PMNs are active participants in the pathophysiology of infarction, exacerbating the tissue damage [61]. In fact, they produce several toxic mediators including oxygen derived free radicals (e. g. superoxide radicals, hydroxyl radicals, hydrogen peroxide) and lipid mediators (e.g. platelet activating polypeptide mediators factor as well as LTs). Main interest lies in cellular adhesion molecules, particularly selectins and integrins, as their antagonists were repeatedly found to diminish neutrophil activation and infarct size [60].

2.2. Leukotrienes and Cerebral I/R Injury

Transient cerebral ischemia initiates a complex series of metabolic events which ultimately lead to neuronal death. After cerebral ischemia, the highly complex pathophysiological process that follows can be separated into 3 successive phases: metabolic stress and excitotoxicity (acute, within hours), inflammation and apoptosis (subacute, hours to days), and repair and regeneration (chronic, days to months)[62, 63]. Post-ischemic inflammation in the subacute phase is an important event in which a large number of cells and molecules/mediators are involved. Among the inflammatory cells, the accumulation of neutrophils and macrophage/microglia in the brain is determinant in the cerebral I/R pathogenesis [64-66].

The importance of LTs in cerebral I/R has been proven by several authors [67-70] who demonstrated that 5-LO expression as well as LT levels are elevated in ischemic brain [71-74]. In particular, in a gerbil model of transient forebrain, it has been reported that, during reperfusion, neurons exhibit dense 5-LO immunoreactivity and the enzyme is redistributed from cytosol to particulate fraction after 3 min reperfusion. Moreover, an increase in LTC_4 levels in all forebrain regions during reperfusion has been observed, although post-ischemic increase was inhomogeneous. In fact, the increase in the hippocampus was greater than in cerebral cortex. Thus, it has been suggested that reperfusion, which was associated with translocation of cytosolic 5-LO to membranes, induced the biosynthesis of LTC_4 that may mediate irreversible reperfusion injury in the hippocampal neurons [71]. An increase in cysLT production has been also observed in rats [68]. In fact, it has been demonstrated in the brain of the rats with focal cerebral ischemia an increase of cysLT production with 2 peaks at 3–24 h and 7 days after middle cerebral artery occlusion and reperfusion. In the late phase (7-14 days) [68] the increased cysLTs are tempospatially related to the astrocytosis in the penumbral region [68]. Other experimental evidences suggest that astrocytes, the predominant cell type in the brain, are affected and contribute to the cerebral ischemic injury [75] through the production of LTs. In fact, it has been reported that the cultured astrocytes produced cysLTs after 1h oxygen-glucose deprivation (OGD)-induced *in vitro* ischemia. Moreover, it has been hypothesized that the released cysLTs might play an autocrine role in the induction of reactive astrocytosis through the interaction with $CysLT_1$ receptor [76]. In particular, *in vitro* ischemia activates astrocytes to produce cysLTs that result in $CysLT_1$ receptor-mediated proliferation and $CysLT_2$ receptor-mediated death [77].

The CysLT receptors, $CysLT_1$ and $CysLT_2$, seem to be the also responsible for the increased blood–brain barrier permeability and for the induced brain edema and neutrophil infiltration observed after cerebral ischemia [72, 78-80]. In fact, $CysLT_1$ receptor mRNA has been detected in the brain [81] and its protein has been found to be primarily expressed in the microvascular endothelium of the human brain tissue [82]. An increase in the expression, spatio-temporally related to acute neuronal injury and late astrocyte proliferation, was also observed in rat brain after focal cerebral ischemia. The role of $CysLT_1$ receptor is also supported by the observation that $CysLT_1$ receptor antagonists, pranlukast (ONO-1078) and montelukast, protected against acute and chronic ischemic brain injury in rats and mice [83-87]. In particular, ONO-1078 possessed a neuroprotective effect on global cerebral ischemia in rats, at least in part related to the inhibition of the up-regulation of vascular cell adhesion molecule 1 (VCAM-1) in different regions of the brain [84].

Supporting evidences have been also reported for $CysLT_2$ receptor [88]. In fact, the expression of $CysLT_2$ receptor mRNA was increased in the rat ischemic core at 6, 12 and 24 h after reperfusion, whereas in the boundary zone after 3, 7 and 14 days, suggesting its involvement in the acute neuronal injury and late astrocyte proliferation in the ischemic brain [88]. The prominent role of CysLT2 in respect to CysLT1 receptor has been proved by the use of neuronal cell line PC12 [89]. In fact, it has been recently demonstrated that transfection with $CysLT_2$, and not with $CysLT_1$ receptor, increased OGD-induced PC12 cell death that was attenuated by the dual $CysLT_1$/$CysLT_2$ receptor antagonist, BAY u9773 [90], suggesting a role of this receptor subtype in ischemic astrocyte and neural cell death [77].

As reported above, recently it has been recently identified a new receptor for CysLTs: GPR17 [31]. This receptor (rat and human) is expressed in the organs typically undergoing ischemic damage, such as brain, heart and kidney. In particular ischemic damage in a rat focal ischemia model was attenuated by inhibition of GPR17, through either CysLT/P2Y receptor antagonists or antisense technology. These results suggest an important role of GPR17 in cerebral I/R [31].

In conclusion, though, in an animal model of focal cerebral ischemia, no difference in cerebral infarct size has been found between 5-LOKO and wild type mice [91], the overall results support the view that LTs are involved in the cerebral I/R injury.

2.3. Leukotrienes and Cardiac I/R Injury

A number of different pathogenetic events may lead to ischemic cardiovascular diseases (ICD), including atherosclerosis, small vessel diseases, cardiac arrhythmias and hypercoagulation. In turn, these conditions are also dependent on a variety of different underlying conditions some of which are inherited whereas others are due to exogenous factors [92]. Most of the treatments available for ischemic insults, including myocardial infarction (MI) and angina, are

directed toward preventing the tissue damage inflicted at the time of reperfusion, when the coronary flow is restored by removing the occlusion. As reported above, genetic factors may contribute to the risk of I/R and recently, gene variants that predispose patients to MI have been reported [6]. In particular, variants of gene involved in LT biosynthesis confer ethnic specific risk of MI. In fact, a haplotype spanning ALOX5AP (FLAP), HapA, was shown to confer risk of both MI and stroke in Iceland [6]. On the contrary this ALOX5AP variant did not associate to ICD in Sweden [93]. Another gene variant in the LT pathway, LTA_4 hydrolase, was subsequently found to confer increased risk to MI, with an approximately 3 fold higher risk in African Americans than in whites [7]. In particular, measurements of LTB_4 production suggest that this risk is mediated through upregulation of the LT pathway [7]. In fact, it has been demonstrated that, in a randomized placebo-controlled phase II trial conducted in patients with MI, LTB_4 is a risk factor of MI and that inhibition of FLAP and LT pathway produces suppression of biomarkers that are associated with MI risk [94]. In contrast, it seems that the ALOX5 (5-LO)-promoter polymorphism not support variation on MI risk [95].

The role of LTs in I/R of the myocardium is also supported by the use of inhibitors of their synthesis. In fact, in a model of coronary artery ligation in rabbit [96] the treatment with BAY X1005, a LT synthesis inhibitor, reduced the mortality rate, protected against the marked electrocardiogram derangement and abolished the significant increase in plasma creatine kinase activity [96]. Likewise, FLM 5011, a 5-LO inhibitor, protected myocardial microvessels against I/R injury after ligation of the left coronary artery in dogs [97].

Other experimental evidences for the involvement of LT in myocardial I/R derive from a direct measurement of LT levels during episodes of MI. In fact, it has been demonstrated that LTB_4 and cysLT levels in systemic artery blood are higher in patients with acute stage of MI and decrease to near-normal control levels by one month after the attack [98]. Of interest, the urinary excretion of LTE_4, the human urinary metabolite of cysLTs, was also increased [99]. In particular, experimental data suggested that LTC_4 is involved more in prolonged than in transient MI [100] and that LTD_4 has a negative inotropic and chronotropic effect in isolated rat hearts with chronic MI [101]. Thus, the evaluation of LTC_4 levels might be useful in clinical diagnosis and management of acute coronary syndromes [102]. Moreover, recently the involvement of the $CysLT_2$ receptor in myocardial I/R injury has been highlighted [103]. In fact, endothelium-targeted over-expression of $CysLT_2$ receptor has been shown to aggravate myocardial I/R injury by increasing endothelial permeability and by exacerbating inflammatory gene expression (VCAM-1 and ICAM) [103].

The involvement of LTs in acute coronary syndrome is also supported by their profound effects on cardiac function, which may be mediated through effects on both coronary blood flow and cardiac contractility [104]. In fact, the blood flow to several vascular beds can be altered by LTs and their synthesis and release may play an important role in the regulation of the peripheral circulation [104]. Moreover, LTs, besides their action on vascular smooth muscle and myocardium, increase the permeability of blood vessels [104].

Although experimental evidence supports a role for LT in myocardial I/R, recently it has been suggested, by the use of 5-LOKO mice, that LTs have no major role in I/R injury in the heart [105].

2.4. Leukotrienes and Pulmonary I/R Injury

Pulmonary I/R injury may result from trauma, atherosclerosis, pulmonary embolism, pulmonary thrombosis and surgical procedures, such as cardiopulmonary bypass and lung transplantation. In particular, pulmonary I/R injury occurs in up to 22% of patients after lung transplantation and is still the main cause of death during the first month after surgery [106].

After pulmonary I/R, cellular injury is accompanied by a rapid remodelling of membrane lipids with the generation of bioactive lipids, such as LTs, that can serve as intra- and/or extracellular mediators. The pivotal role of LTB_4 in the pulmonary I/R injury has been valued by several experimental data. In particular, neutrophil accumulation in the lung has been associated with pulmonary I/R that leads to respiratory failure [107, 108]. It has been suggested that the inflammatory cell accumulation was caused by LTB_4 generated by the ischemic tissue and released into the circulation [109]. In fact, LTs mediate neutrophil sequestration and lung edema after hindlimb ischemia [109]. Moreover, it has been reported that the leukocytes from transgenic mice, overexpressing the LTB_4 receptor, showed an increased PMN trafficking to lungs after I/R [110], whereas 5-LOKO mice showed lower PMN infiltration in reperfused lungs in comparison to 5-LOWT mice [110]. An increase of LTB_4 levels in bronchoalveolar lavage fluid after I/R injury has also been observed in rat I/R lung injury model, [111, 112], as well as in serum after pulmonary I/R injury in dogs [113]. Moreover, the role of LTs is suggested by reports showing that after limb ischemia-induced lung injury [114] there is an increase in LTB_4 plasma levels as well as in LTC_4 blood and bronchoalveolar lavage fluid level [115].

2.5. Leukotrienes and Renal I/R Injury

Clinically, renal I/R occurs in a variety of medical and surgical settings and is responsible for the development of ischemic acute renal failure and acute tubular necrosis, e.g. in renal transplantation where I/R of the kidney directly influences graft and patient survival. Multifactorial processes are involved in the development and progression of renal I/R injury, among them, renal inflammation, involving cytokine/adhesion molecule cascades, with recruitment, activation, and diapedesis of circulating leukocytes, and chemotactic mediators [116], such as chemokines and 5-LO metabolites [117, 118].

The involvement of LT in renal I/R has been demonstrated by the use of the 5-LO inhibitor, zileuton, which reduced renal dysfunction and injury caused by bilateral occlusion and reperfusion of mouse kidneys [119]. The compound also abolished the significant increase in plasma levels of LTB_4 observed in wild-type mice after the bilateral renal I/R. Moreover, the degree of renal dysfunction, injury and inflammation was reduced in 5-LOKO mice further supporting that 5-LO and its metabolites contribute to the pathophysiology of renal I/R injury. In particular, both in mice treated

with zileuton and in the corresponding 5-LOKO we observed, after I/R, a reduced expression of ICAM-1 in the kidney that corresponded to a diminished PMN accumulation. This is not entirely surprising given that the 5-LO metabolite, LTB_4, is a potent chemokine. Similar results demonstrated that LTB_4 alone appears sufficient to cause cells to migrate into post ischemic renal tissue supporting the role for this LT as an important mediator in the pathophysiology of renal dysfunction caused by kidney I/R [120].

The role of cysLTs in renal I/R has been also investigated by the use of receptor antagonists [121]. It has been demonstrated that $CysLT_1$ receptor antagonist, montelukast, reversed I/R-induced oxidant responses and improved microscopic damage and renal function. It seems likely that montelukast protects kidney tissue by inhibiting neutrophil infiltration, balancing oxidant-antioxidant status and regulating the generation of inflammatory mediators [121].

2.6. Leukotrienes and Intestinal I/R Injury

Intestinal I/R injury is generally the result of arterial occlusion by thrombi or emboli and, more frequently, by non occlusive processes, like acute mesenteric ischemia, small bowel transplantation, abdominal aortic aneurysm, severe burns and hemorrhagic, traumatic or septic shock [122]. I/R injury of the gastrointestinal tract, associated with haemorrhage and other shock states, is characterized by a number of microvascular and mucosal alterations, including endothelial cell swelling, capillary plugging, a prolonged reduction in gastrointestinal blood flow and mucosal barrier dysfunction [123]. An important component of intestinal I/R is endothelial dysfunction [124, 125] especially attributed to activated adherent PMNs [126]. In fact, I/R is a stimulus for leukocyte-endothelial cell interaction and migration into tissues.

It has been demonstrated that LTB_4 plays a pivotal role in endothelial dysfunction occurring in splanchnic artery occlusion shock (SAO), in the rats, by chemoattraction and activation of neutrophils on the surface of vascular endothelial cells [127]. Moreover, it seems that the activation of BLT receptor plays a minor role in the local, remote and systemic injuries following severe intestinal I/R in rats [128]. The role of 5-LO metabolites has been also investigated by the use of 5-LOKO mice. In fact, we have demonstrated that 5-LO mediates leukocyte-endothelial cell interactions by regulating the expression of P-selectin, E-selectin and ICAM-1 during SAO in 5-LOKO animals [58]. Interestingly, the down-regulation of the adhesion molecules, in the intestine as well as in the lung tissue of SAO-shocked 5-LOKO mice, was associated with the reduction of leukocyte infiltration.

The role of LTs is also supported by results demonstrating that LTB_4 and LTC_4 levels triple after I/R in the canine intestinal mucosa [129] and that LTC_4 regulates the splanchnic blood flow during mild haemorrhage/reperfusion injury in rats [130]. More generally, LTs seem to play a significant role also in other experimental models of I/R intestinal injury, like in a canine model of hypothermic I/R injury [131] and in hypotension associated with I/R of the small intestine in rats [132]. In particular, in this model the inhibition of LT biosynthesis by zileuton significantly improved reperfusion, intestinal blood flow and VO_2, and abolishes the I/R-induced increase in mucosal neutrophil infiltration in normothermic I/R injury [129].

Recently, it has been demonstrated that splanchnic I/R in rats activates gut PLA_2-mediated release of AA into the lymph where it is delivered to the lungs, provoking LTB_4 production and subsequent PMN-mediated lung injury [133].

2.7. Leukotrienes and Hepatic I/R Injury

Hepatic injury secondary to I/R is an important clinical issue. It has been implicated in the pathogenesis of a variety of clinical conditions including trauma, thermal injury, hypovolemic and endotoxin shock, reconstructive vascular surgery, liver transplantation and liver resection surgery [134-139]. Possible consequences of hepatic I/R injury include liver failure and/or multi-organ system failure, resulting in morbidity and mortality [140]. Extensive investigations during the past decade suggest that an inflammatory response and microcirculatory disturbances contribute to reperfusion injury and cause, in severe cases, liver failure [139, 141, 142]. It has been reported that LTs are associated with several liver injury such as fulminant hepatitis [143], liver cirrhosis [144], cholestasis, hepatic inflammation, portal hypertension, hepatorenal syndrome, fulminant hepatic failure, primary graft nonfunction following liver transplantation [145, 146] and hepatic I/R injury [147]. In particular, a 4- to 5-fold increase of the cysLTs content in the hepatic tissue after 12 and 24 h reperfusion, accompanied by the enhancement of hepatic edema and plasma ALT elevation, has been observed [148]. According to recent findings LTC_4 accumulation in rat liver subjected to I/R may be partially caused by up-regulation of LTC_4S expression and by the increase of LTC_4 synthesis enzyme and/or activities [149]. However, since the pathophysiology of hepatic I/R injury is so complicated, it is essential to further study the mechanisms responsible for LTC_4 accumulation in hepatic I/R injured rats [149].

Contrasting data have been reported on LTB_4 involvement in hepatic I/R injury. In fact, either an increase or no change in LTB_4 levels have been observed after hepatic I/R injury [150]. Therefore, the involvement of LTB_4 needs further investigations.

3. INVOLVEMENT OF LEUKOTRIENES IN THE PATHOGENESIS OF SHOCK

3.1. Shock

Sepsis is a complex pathophysiological response of the body to a systemic infection and may result in severe disorders such as septic shock, characterized by hypotension, hypothermia, poor tissue perfusion and multi organ dysfunction syndrome (MODS) [151, 152]. In particular, severe sepsis is defined as the presence of sepsis and one or more organ dysfunctions (acute lung injury; coagulation abnormalities; thrombocytopenia; altered mental status; renal, liver, or cardiac failure; hypoperfusion with lactic acidosis) [153, 154], while septic shock is defined as the presence of sepsis and arterial hypotension [155]. The mortality rate of sepsis may range from 30% to 50% for severe cases [156].

The inflammatory response is a central component of sepsis as it drives the physiological alterations that are recognized as the systemic inflammatory response syndrome

(SIRS) [157]. A successful inflammatory response eliminates the invading microorganisms without causing lasting damage, however sepsis develops when the initial appropriate host response to an infection becomes amplified and then aberrant. Many scientists believe that sepsis develops as a result of production of several mediators such as pro-inflammatory molecules (TNF-α, IL-1, IL-6 and IL-8), lysosomal enzymes, superoxide-derived free radicals, vasoactive substances and eicosanoids [prostaglandins (PGs) and LTs] [158]. Moreover, the vascular changes in septic shock [159] have been interpreted on the basis of the effects of inflammatory mediators on the vascular endothelium [160]. In fact, the initial responses to endotoxemia are detectable in the microcirculation as a microvascular inflammatory response characterized by activation of the endothelium stimulating these cells from their normal anticoagulant state to a procoagulant state with increased adhesiveness for platelets and leukocytes. The infiltration and accumulation of PMNs represent a crucial event for the development of secondary organ and tissue damage [161-164]. Moreover, leukocyte/endothelial cell interaction is also induced by the generation of pro-inflammatory mediators, such as LTs, which up-regulate adhesion molecule expression [165-167]. It has therefore been proposed that during human sepsis a widespread endothelial damage and death occurs which leads to MODS.

3.2. Leukotrienes and Septic and Non Septic Shock

LT generation in sepsis may serve as a biomarker for survival in the critical ill [168] since a negative correlation between the ability of blood mononuclear cells to synthesize LTC_4 and mortality in septic patients has been identified. In fact, a significantly increased mortality rate has been observed [169] in septic patients, whose *in vitro* stimulated pooled blood mononuclear cells were unable to produce increased amount of LTC_4 over time. Moreover, [170], it has been reported that in sepsis the PG basal levels in white blood cells (WBC) were significantly decreased in comparison to healthy subjects, whereas isolated WBC stimulated with AA have the same capacity to generate PG. In contrast, LT levels were significantly higher in septic patients than in healthy subjects, but WBC were able to produce, after stimulation with AA, greater amount of LTs only in patients who survived the septic state. The authors suggested that this inability of WBC to further enhance LT production during the course of sepsis in non-survivors could be explained as part of the "immunoparalysis" seen during sepsis associated with bad outcome. Thus, the functional analysis of LTs and PGs during sepsis might turn out to be a suitable approach for the estimation of the future course of the disease [170].

Moreover, a complex dynamic equilibrium between PGs and LTs in septic shock has been suggested. In fact, PGI_2 blockade during bacteraemia significantly increased LT production that on the contrary was decreased by PGI_2 infusion, suggesting that endogenous PGI_2 may blunt LT release during septic shock [171]. These data indicate that LT production can also be regulated by PGs.

The immunopathogenesis of sepsis is characterized by an overwhelming suppressed adaptive immunity [172, 173]. Although activation of the innate immune system by microbial pathogens and their products was reported to contribute to hyper-inflammation and organ injury during systemic inflammatory responses, many aspects of sepsis immunopathogenesis need further elucidation.

The overall results concerning the role of LTs in the immune response demonstrate that they have divergent effects during the induction and evolution of septic shock. Initially, they participate in local innate immune control. However, if the severity of infection overwhelms local immunity and microbial dissemination ensues, cysLTs contribute to the deleterious effects on the vasculature, resulting in vascular leak, hypotension and inadequate tissue perfusion [174]. In fact, a reduction in peritoneal neutrophil accumulation and an increase in the number of bacteria in the peritoneal cavity has been demonstrated, in different models of peritonitis with severe sepsis (such as a cecal ligation and puncture), in 5-LOKO mice. Despite this impairment of local innate immunity, the null mice exhibited a marked improvement in survival, and this protection was also seen in wild-type animals treated with the LT synthesis inhibitor, MK 886. A survival advantage in severe sepsis was also observed in mice treated with the CysLT receptor antagonist, MK 571, but not with the LTB_4 receptor antagonist, CP 105 [174]. Moreover, in the 5-LOKO mice a reduced vascular leak and serum lactate levels was observed, whereas treatment of wild-type mice with MK 571 produced a less sepsis-induced hypotension [174]. These data demonstrate opposite effects of cysLTs on innate immune *vs* hemodynamic responses, demonstrating protective effects on local immunity and deleterious effects on the vasculature [174]. In particular, cysLTs have long been recognized to increase microvascular permeability in various organs [175-178]. Thus, although LTs initially play a protective role during septic shock development, they play a detrimental role, indicating salutary effects of LT inhibitors and antagonists in endotoxin shock [179]

Septic shock induced by the injection of endotoxin is widely used in animal experimental models, and hypothermia is one of the prominent features of the acute phase response to lipopolysaccharide (LPS) [180]. It has been demonstrated that MK 886 significantly attenuated the hypothermia induced by LPS and significantly reduced the elevation in hypothalamic LT production in the rats [180]. Moreover, in a model of rat endotoxic shock, the treatment with BW A137C, a 5-LO inhibitor, attenuated acute microvascular injury produced by LPS [181]. Similarly, the administration of MK 886 attenuated the hypotension and partially reversed the impaired vascular responsiveness observed in a rabbit model of endotoxic shock [182] as well as blocked the toxin-induced coronary vasoconstrictor response and the loss of rat myocardial contractility [183]. Thus, the overall results show that LTs cause pulmonary hypertension, systemic hypotension [184], hypothermia [185] and an increased vascular permeability during bacteraemia [184].

As previously reported, septic shock can develop into MODS, with lung as the first organ involved. In particular, LTs have been implicated as possible mediators of endotoxin-induced acute lung injury. In fact, the presence of LTs in the bronchoalveolar lavage fluid of patients with sepsis as well as an increase of cysLT levels in lung tissue of endotoxin-challenged rodents, has been reported [186, 187].

Moreover infusion of LTs into animals produced an acute lung injury resembling the clinical presentation of endotoxemia, which includes pulmonary hypertension and increased vascular permeability, resulting in pulmonary oedema and hypoxemia [188, 189].

Another target organ during sepsis is the liver, which is continuously exposed to endotoxin *via* the portal circulation. Liver sinusoidal cells, in particular macrophages and endothelial cells play an important role in clearance of endotoxin from the blood [190]. Excessive levels of endotoxin can readily overcome this clearance mechanism leading to liver damage [191]. The release of oxidants eicosanoids and cytotoxic pro-inflammatory cytokines by LPS-activated macrophages and endothelial cells seems to be in part implicated in this process [192-194]. In fact, it has been demonstrated that 5-LO mRNA expression is increased after endotoxin administration in liver endothelial cells providing additional support for the idea that LTs play a role in recruitment and activation of leukocytes into liver tissue during acute endotoxemia [195]. Moreover, we have demonstrated in collaboration with Thiemermann and coworkers [56], that lung, liver, ileum, renal and pancreatic dysfunction and injury, caused by endotoxemia, as well as PMN infiltration in the lung and ileum, were reduced in rats treated with 5-LO inhibitor zileuton and in 5-LOKO mice. Zileuton also reduced the LPS-induced expression of β2 integrins CD11b/CD18 on rat leukocytes. Thus zileuton seems to protect organs against endotoxin-induced dysfunction and injury by inhibiting the LT synthesis, thereby reducing the LT-induced stimulation of β2-integrin-dependent adhesion and the subsequent recruitment of neutrophils [56].

It is known that MODS remains a principal cause of death after severe shock or trauma, not only in the presence, but also in the absence of sepsis [196-202]. A marked biosynthesis of LTC_4 and LTB_4 was observed also in an experimental model of MODS induced by non septic shock (zymosan-induced peritonitis and MODS) [203]. Other results support the pivotal role of LTs in zymosan-induced peritonitis model. In fact, oedema associated with zymosan-induced peritonitis was markedly reduced in animals lacking FLAP [204] and it has been reported that 5-LO inhibitors and LTB_4 receptor antagonists are effective in preventing the development of organ failure since they reduce neutrophil infiltration [203]. Same results were obtained by the use of LTA_4 hydrolase-deficient mice [205] and 5LOKO mice [57]. Interestingly, a reduced expression of adhesion molecules, such as P-selectin and ICAM-1 in the lung and ileum was observed. Thus, as for septic shock, these results demonstrate that LTs exert a role in zymosan-induced non septic shock by the regulation of neutrophil recruitment both at the rolling and firm adhesion phase [57].

Similar data were obtained in another model of non septic induced-MODS [206]. In fact, it has been demonstrated that chronic renal failure-induced multiple-organ injury in rats is alleviated by the selective $CysLT_1$ receptor antagonist montelukast [206]. In particular, protective effects of montelukast on chronic renal failure-induced injury were attributed to its ability to inhibit neutrophil infiltration and apoptosis, to balance oxidant-antioxidant status and to regulate the generation of pro-inflammatory mediators [206]. Moreover, it has been observed an increase in pulmonary LTB_4 production within the lung also in an experimental model of traumatic brain injury that is known to cause several secondary effects, which lead to MODS [207].

4. SUMMARY

This review discusses the role of LT pathway in various forms of I/R injury (cerebral, cardiac, pulmonary, renal, in-

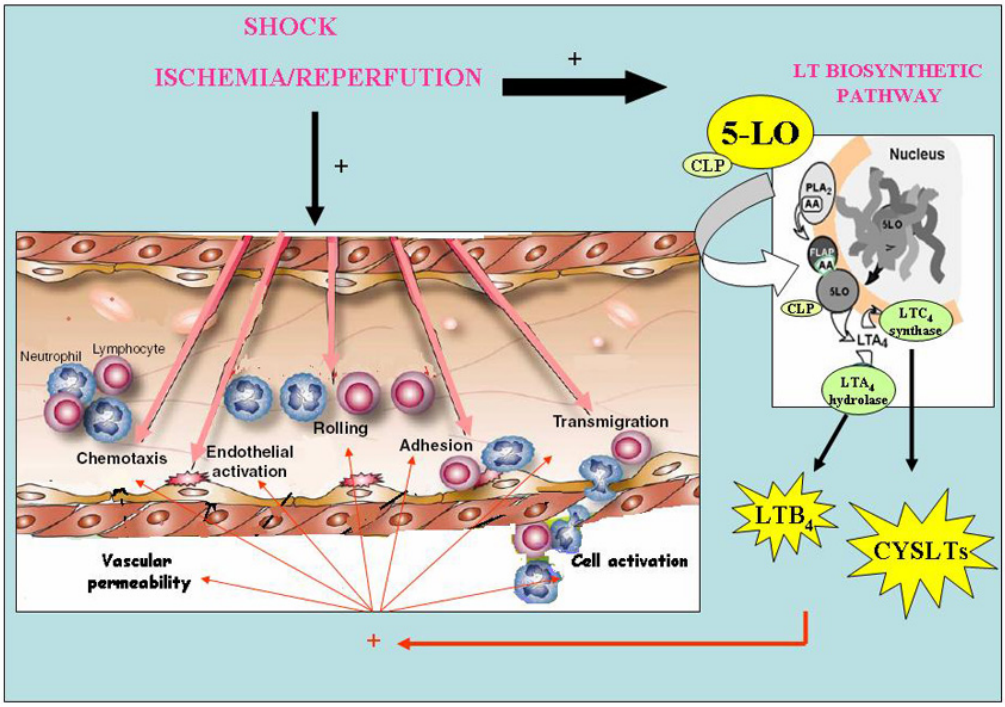

Fig. (1). Proposed scheme for the role of LT pathway in I/R injury and shock.

testinal and hepatic) and shock (septic and non septic), and delineates the evidence supporting the potential beneficial utility of anti-LT therapy (5-LO inhibitors and LT receptor antagonists) as a promising new approach to limit I/R-and shock-induced tissue damage.

There is a marked activation of LT biosynthetic pathway in various forms of I/R and shock, which correlates with the degree of tissue injury and inflammation. In particular, accumulating evidences suggest that anti-LT therapy may protect against organ I/R injury by decreasing the endothelial cell activation/inflammatory response, vascular permeability and recruitment, adhesion and activation of inflammatory cells by the inhibition of the adhesion molecule expression (Fig. (**1**)).

A better understanding of the mechanisms underlying the role of the 5-LO enzyme and/or its metabolites, in particular LTs, in the regulation of neutrophil trafficking, might open new perspectives in the treatment of organ damage associated with shock and I/R injury.

ABBREVIATIONS

5-HPETE = 5-Hydroperoxyeicosatetraenoic acid

5-HETE = 5-Hydroxyeicosatetraenoic acid

5-LO = 5-Lipoxygenase

FLAP = 5-Lipoxygenase-activating protein

AA = Arachidonic acid

cysLT = Cysteinyl LT

$cPLA_2$ = Cytosolic phospholipase A_2

CLP = Coactosin-like protein

ERK = Extracellular signal-regulated kinase

GPR17 = G protein–coupled receptor 17

I/R = Ischemia and reperfusion

ICD = Ischemic cardiovascular diseases

KO = Knockout

LTs = Leukotrienes

LTB_4 = Leukotriene B_4

LTC_4S = Leukotriene C_4 (LTC_4) synthase

LTD_4 = Leukotriene D_4

LTE_4 = Leukotriene E_4

LPS = Lipopolysaccharide

MAPK = Mitogen-activated protein kinase

MODS = Multi organ dysfunction syndrome

MI = Myocardial infarction

PMNs = Polymorphonuclear leukocytes

PGs = Prostaglandins

SAO = Splanchnic artery occlusion shock

SIRS = Systemic inflammatory response syndrome

VCAM-1 = Vascular cell adhesion molecule 1

WBC. = White blood cells

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