New Insights into the Regulation of Liver Inflammation and Oxidative Stress

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Abstract: Pro-inflammatory lipid mediators (i.e. eicosanoids), cytokines (i.e. TNF-α) and reactive oxygen species are targets of interest in the regulation of liver inflammation and oxidative stress. In the current review, we summarize recent advances in the pharmacological modulation of these pathways with especial emphasis on the participation of Kupffer cells, the liver resident macrophages and the cell type most directly related to the production of inflammatory mediators in this organ.

Key Words: Cyclooxygenase, 5-lipoxygenase, TNF-α, TGF-β, reactive oxygen species.

INTRODUCTION

There is growing recognition of the importance of inflammation in initiating the sequence of events leading to liver injury. Following an insult of any etiology, the liver develops a localized inflammatory response, which serves to destroy, dilute or wall off the injurious agent and the injured tissue. If the insult persists or the inflammatory response remains uncontrolled or is not properly resolved, this response becomes chronic and ultimately leads to the formation of tissue scar, fibrosis and eventually cirrhosis [1-3]. Among the different mediators involved in inflammation, arachidonic acid-derived lipid mediators (i.e. eicosanoids) as well as cytokines (i.e. TNF-α) play a prominent role. On the other hand, reactive oxygen species play a pivotal role in the development of oxidative stress, which is another common mechanism closely related to inflammatory response and is involved in the progression of liver disease. Both inflammation and oxidative stress represent two targets of interest to halt the progression of liver diseases. In the current manuscript, we review the most recent advances in the current knowledge of the relationship between inflammation, oxidative stress and liver disease, and discuss some potential pharmacological interventions of these pathways, with especial emphasis on the contribution of Kupffer cells, the liver resident macrophages.

THE ARACHIDONIC ACID CASCADE

The arachidonic acid cascade comprises a number of small lipid mediators originating from the oxidation of arachidonic acid, an essential ω-6 polyunsaturated fatty acid. In mammals, eicosanoid biosynthesis is usually initiated by the activation of phospholipase A_2 and the release of arachidonic acid from membrane phospholipids in response to the interaction of a stimulus with a receptor on the cell surface [4]. There are two classical routes of oxidation of free arachidonic acid in mammalian cells: the cyclooxygenase (COX) pathway that results in the formation of prostaglandins (PGs) and thromboxane (TX); and the lipoxygenase (LO) pathway that catalyzes the formation of leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs) (Fig. (**1**)). Alternatively, arachidonic acid can be transformed by the cytochrome P450 "epoxygenase" pathway to epoxyeicosatrienoic acids [5]. The COX and LO pathways are of clinical relevance in inflammatory diseases because they represent the primary targets for non-steroidal anti-inflammatory drugs (NSAIDs), selective COX-2 inhibitors (COXIBs) and the so-called leukotriene-modifying drugs, respectively [reviewed in references 6-8].

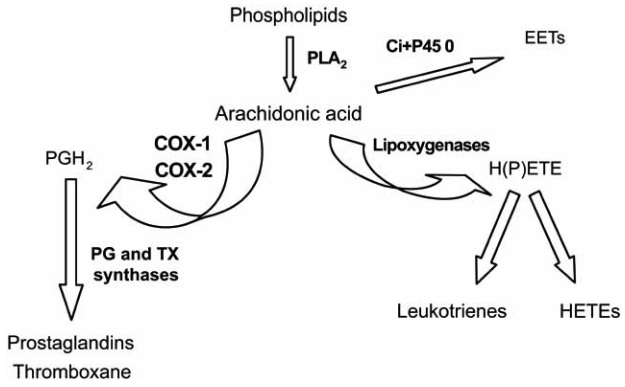

Fig. (1). The eicosanoid cascade. Arachidonic acid is converted into prostaglandins (PGs) and thromboxane (TX) by the cyclooxygenase (COX) pathway and into leukotrienes and HETEs by the lipoxygenase pathway. Arachidonic acid is also transformed by the cytochrome P450 to epoxyeicosatrienoic acids (EETs). PLA_2: Phospholipase A_2.

The Cyclooxygenase (COX) Pathway

COX is the key enzyme in the biosynthesis of PGs from arachidonic acid [9,10]. COX is a membrane-bound bifunctional enzyme that catalyzes the first two committed steps, namely cyclooxygenation and peroxidation, in the pathway leading to the formation of PGs and TX. In fact, COX is a dual enzyme that adds two oxygen molecules to arachidonic acid to form PGG_2 and subsequently reduces this

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cycle hydroperoxide to the highly reactive endoperoxide PGH_2 [9,10]. PGH_2 functions as an intermediate substrate for the biosynthesis of PGs of the E_2, F_2 and D_2 series and also of PGI_2 (prostacyclin) and TXA_2 by specific synthases (Fig. (**2**)). The coupling of PGH_2 synthesis to its transformation to PGs and TX by downstream enzymes is intricately orchestrated in a cell-specific fashion, so that any given prostanoid-forming cell tends to form only one of these compounds as its major product. For example, PGH_2 is converted to PGD_2 by the cytosolic enzyme PGD synthase in brain and mast cells. PGH_2 can alternatively be converted to $PGF_{2\alpha}$ by PGF synthase, which is mainly expressed in the uterus. Vascular endothelial cells produce PGI_2 or prostacyclin from PGH_2 by means of PGI synthase, and platelets release TXA_2 from PGH_2 through the action of TX synthase. Both PGI_2 and TXA_2 have a very short half-life (30 seconds and 3 minutes, respectively) and are rapidly hydrolyzed to the inactive compounds 6-keto-$PGF_{1\alpha}$ and TXB_2 (Fig. (**2**)). Finally, PGE_2 is

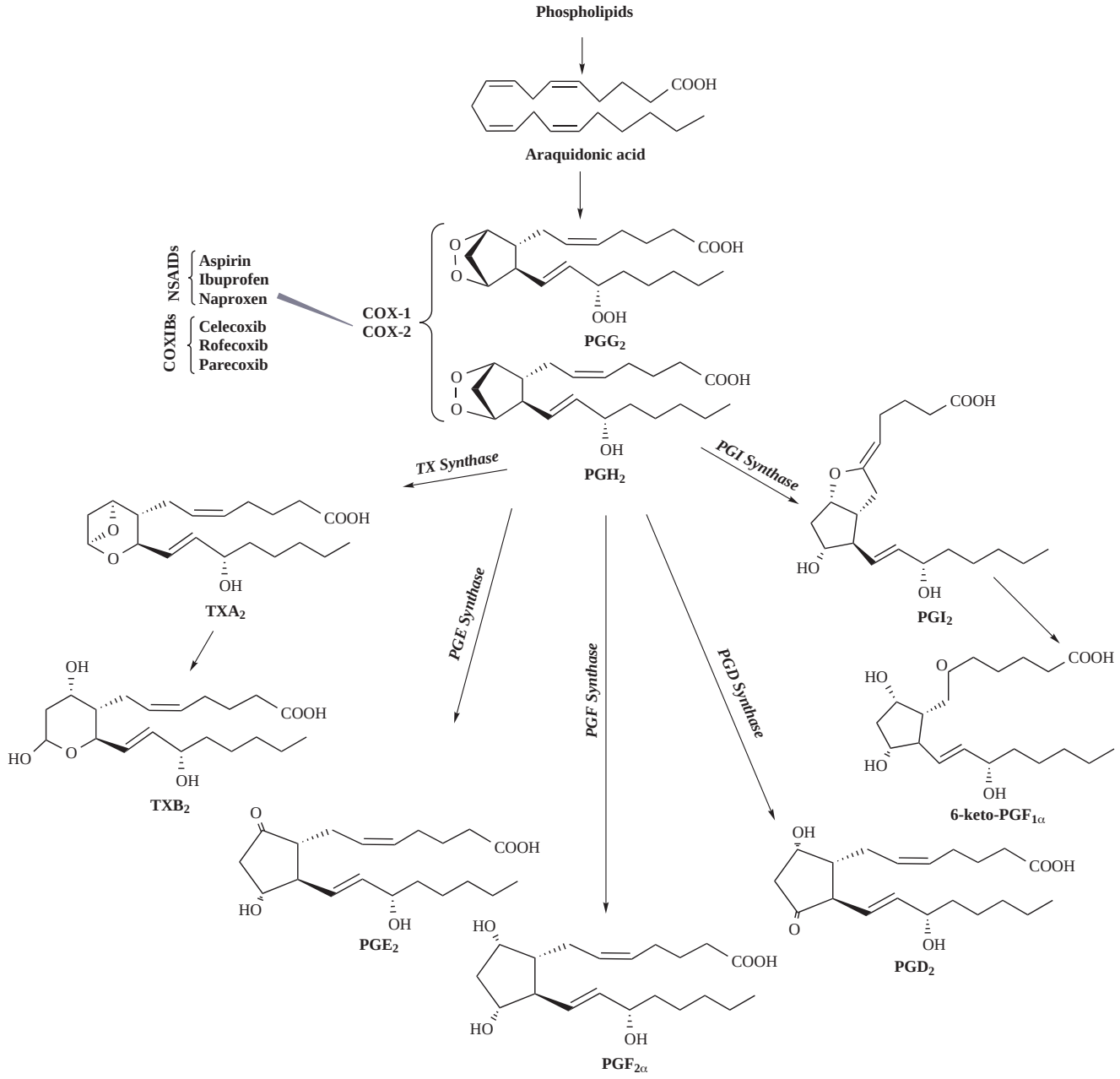

Fig. (2). The cyclooxygenase (COX) pathway. COX exists in two different isoforms (COX-1 and 2) and oxygenates arachidonic acid to form prostaglandin (PG) G_2 that is further reduced to PGH_2. PGH_2 is a highly unstable endoperoxide that is rapidly converted by specific synthases to PGs of the E_2, F_2 and D_2 series and also to PGI_2 (prostacyclin) and thromboxane (TX) A_2. Both PGI_2 and TXA_2 have a very short half-life and are rapidly hydrolyzed to the inactive compounds 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively. NSAIDs and selective COX-2 inhibitors (COXIBs) pharmacologically modulate the COX pathway.

formed in many cell types by enzyme PGE synthase (PGES), which may be found under three different isoforms in mammals: mPGES-1, cPGES-1 and mPGES-2. mPGES-1 was first identified and characterized by Jakobsson *et al.* [11] in 1999 as a member of the membrane-associated proteins involved in the eicosanoid and glutathion metabolism (MAPEG) superfamily and has the ability to catalyze the conversion of PGH_2 to PGE_2. Later on, cPGES-1, an ubiquitously expressed cytosolic form of PGES, which also isomerizes PGH_2 to PGE_2 rather specifically in the presence of gluthation, was also cloned [12,13]. Finally, mPGES-2, a second isoform of membrane-associated PGES, was identified in 2002 [14]. Among the three PGES enzymes, mPGES-1 has received more attention because this enzyme is inducible and functionally linked with COX–2 [13,15]. PGs, mainly PGE_2, play a key role in the development of the five cardinal signs of inflammation: *edema*, *erythema*, *pain*, *fever* and *loss of function*. PGE_2 increases vascular permeability contributing to fluid extravasation and the appearance of *edema* (*swelling*), in a synergistic fashion with other soluble factors such as complement, bradykinin, histamine and LTs [8]. In addition, PGE_2 is a potent vasodilator that increases tissue blood flow, contributing to the appearance of the characteristic *erythema* (*redness*) [16]. On the other hand, PGE_2 sensitizes peripheral sensory nerve endings located at the site of inflammation and acts in the spinal cord to evoke hyperalgesia, *pain* [17,18]. Finally, PGE_2 is crucial in the appearance of *fever* [19]. *Pyresis* is the consequence of increased levels of PGE_2 in the central nervous system secondary to the actions of the pro-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) produced by activated immune cells in the systemic circulation [20]. All of these actions of the PGs lead to a loss of function. It is also important to mention the ability of PGs to potentiate and prolong the action of other mediators of inflammation such as bradykinin, histamine, neurokinins and complement [8]. The observation that mice lacking mPGES-1 gene show impaired inflammatory, pain and fever responses provides a clearly proof of the important role of PGE_2 in inflammation [21,22]. In mammalian cells, there are two COX isoforms: COX-1, which is constitutively expressed, and COX-2, which is inducible [23-25]. COX-2 is increased during the inflammatory process and is involved in the synthesis of PGs in inflammation. In fact, COX-2 gene expression is up-regulated by soluble inflammatory mediators (IL-1α and β, TNF-α, interferon (IFN)γ and LPS), growth factors (epidermal growth factor, platelet-derived growth factor and fibroblast growth factor) and oncogenes (v-src and v-ras) [26].

Drugs affecting PG formation: NSAIDS and COXIBs

In 1971 Vane [27], Ferreira *et al.* [28] and Smith *et al.* [29] published their seminal observations linking the ability of NSAIDs to suppress inflammation to the inhibition of COX and PG biosynthesis. NSAIDs are among the most widely prescribed class of pharmaceutical agents worldwide, having broad clinical utility in treating pain, fever and inflammation [30]. Unfortunately, apart from their beneficial anti-inflammatory, antipyretic and analgesic effects, NSAIDs also exert unwanted side-effects, particularly in the gastrointestinal tract [31,32]. Gastroduodenal ulceration is the best characterized serious adverse event of NSAID therapy and is the consequence of inhibiting the biosynthesis of PGs, which are the most important gastric cytoprotective agents [31,32]. Since COX-1-derived PGs are presumably involved in housekeeping functions including gastrointestinal cytoprotection, and COX-2-derived PGs are implicated in inflammation, the gastrotoxicity associated with the administration of traditional NSAIDs (including aspirin, indomethacin, ibuprofen and meclofenamate) is considered to be the consequence of the inhibition of both COX-1 and COX-2. That is, at the concentrations required to inhibit PG biosynthesis at the sites of inflammation (COX-2 activity), NSAIDs also elicit a marked suppression of PG production in the gastrointestinal and renal systems (COX-1 activity). After the discovery of COX-2 and the characterization of its role in inflammation, it became evident that the lower range of deleterious effects exerted by some existing NSAIDs, including etodolac (*Lodine®*), meloxicam (*Mobic®*) and nimesulide (*Mesulid®* and others) was due to their higher selectivity for the COX-2 isoform. However, the most important advance in the field of COX inhibition occurred when drug companies took up the search for a new class of compounds specifically designed to selectively inhibit COX-2, without affecting the COX-1-dependent PG biosynthesis [33,34]. The first generation of selective COX-2 inhibitors displayed high selectivity for blocking COX-2 activity *in vitro* and proved to be as efficacious as standard NSAIDs in a number of *in vivo* models of inflammation (rat carrageenan-induced foot-pad edema and rat adjuvant-induced arthritis) and hyperalgesia (rat carrageenan-induced hyperalgesia) [33,35,36]. These results led to the rational design of the first clinical trials for selective COX-2 inhibitors, which were sufficient to prove that these compounds provide significant relief of the signs and symptoms of osteoarthritis and rheumatoid arthritis and alleviate pain following dental extraction, while reducing the incidence of gastrointestinal ulcers and erosions seen with standard NSAID therapy [37-41]. This novel class of compounds is of particular interest for combating inflammation in diseases such as cirrhosis with ascites, in which renal function is critically dependent on COX-1-derived PGs [42-45]. The two selective COX-2 inhibitors first approved and marketed were celecoxib (*Celebrex®*) and rofecoxib (*Vioxx®*). A second generation of selective COX-2 inhibitors including valdecoxib (*Bextra®*), etoricoxib (*Arcoxia®*), parecoxib, an injectable prodrug of valdecoxib (*Dynastat®*), and lumiracoxib (*Prexige®*) have also been approved for treatment of osteoarthritis, rheumatoid arthritis, primary dysmenorrhea and postoperative pain (*Bextra®, Arcoxia®, Dynastat®* and *Prexige®* are approved in Europe whereas only *Bextra®* is approved in the US). Since their introduction into the market in 1999, selective COX-2 inhibitors have become hugely popular and one of the world's best selling drug class. Unfortunately, *Vioxx®*, was recently withdrawn from the market because long-term use was associated with a higher incidence of thrombotic events [46]. More recent studies have unearthed the fact that increased risk of cardiovascular events is common with most COXIBs and NSAIDS [47,48].

5-Lipoxygenase (5-LO) Pathway

Arachidonate 5-LO is the key enzyme in the biosyntesis of LTs. It transforms free arachidonic acid to 5-HpETE through the stereospecific abstraction of the pro-*S* hydrogen

at carbon-7, followed by insertion of molecular O_2 at carbon-5 [49]. 5-HpETE is further reduced to either 5-HETE or subjected to the stereospecific removal of the pro-*R* hydrogen at carbon-10 to generate the highly unstable allylic epoxide LTA_4 [50]. Once formed, LTA_4 is rapidly transformed to either LTB_4 *via* stereoselective hydration by LTA_4 hydrolase [51] or to LTC_4 through glutathion conjugation catalyzed by LTC_4 synthase [52] (Fig (**3**)). Sequential metabolic reactions catalyzed by γ-glutamyl transferase and a specific membrane-bound dipeptidase, convert LTC_4 into LTD_4 and LTE_4, respectively (Fig. (**3**)). Together LTC_4, D_4 and E_4 are termed cysteinyl-leukotrienes (Cys-LTs) and in the past were referred to as the slow-reacting substances of anaphylaxis. Upon cellular activation, cytosolic or nuclear 5-LO translocates to the nuclear envelope where it interacts with phospholipase A_2, which makes free arachidonic acid available to 5-LO [53]. 5-LO also interacts in the nuclear envelope with 5-LO activating protein (FLAP), a resident integral protein which functions as an arachidonic acid transfer protein facilitating the binding of arachidonic acid to 5-LO [53,54]. Both 5-LO translocation and FLAP are crucial for the biosynthesis of 5-LO-derived products [53-56]. LTs exert their biological effects *via* activation of G-protein coupled receptors (GPCRs). To date, two LTB_4 and two Cys-LT receptors have been cloned [57-60]. The B-LT_1 receptor and the recently characterized B-LT_2 receptors bind LTB_4 with high and low affinities, respectively. The B-LT_1 receptor is mainly located on leukocytes and its activation elicits a remarkable chemotactic response, whereas the B-LT_2 receptor displays a widespread tissue distribution pattern the function of which is currently unknown [57,58]. The two types of Cys-LT receptors, Cys-LT_1 and Cys-LT_2, bind LTC_4 and LTD_4. Cys-LT_1 is found in

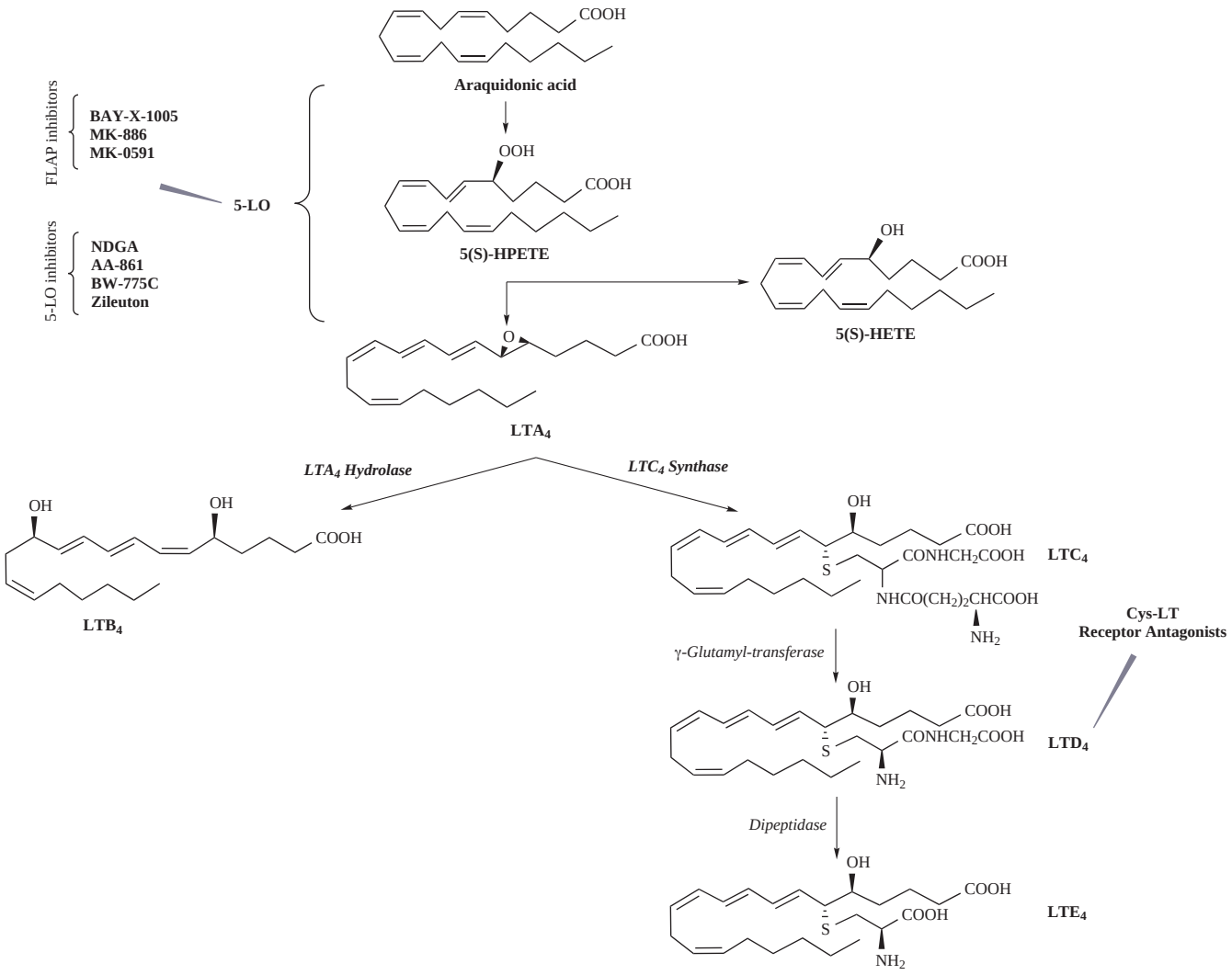

Fig. (3). The 5-lipoxygenase (5-LO) pathway. 5-LO catalyzes the oxygenation of arachidonic acid to the highly unstable allylic epoxide leukotriene (LT) A_4. LTA_4 is either hydrolyzed to LTB_4 by a specific LTA_4 hydrolase or converted into LTC_4 by the addition of the peptide glutathion by a specific LTC_4 synthase. LTC_4 can undergo further metabolism through a series of peptidic cleavage reactions to yield LTD_4 and LTE_4. The clinically relevant LT-modifying drugs include 5-LO and five lipoxygenase activating protein (FLAP) inhibitors as well as Cys-LT_1 receptor antagonists.

airway smooth muscle cells and vascular endothelial cells and its activation promotes vasoconstriction and cell adherence [59]. Cys-LT_2 is distributed within the pulmonary veins, spleen, Purkinje fibers of the heart and the adrenal gland and its function remains unknown [60]. The 5-LO pathway leading to LT formation is intricately involved in allergic and inflammatory reactions. Indeed, 5-LO is mainly expressed in inflammatory cells such as polymorphonuclear leukocytes, eosinophils, monocyte/macrophages, mast cells, dendritic cells and B-lymphocytes [6]. On the other hand, LTs are highly potent mediators of inflammation. LTB_4 induces endothelial adhesion, chemotaxis and activation of leukocytes, lysosomal enzyme secretion and superoxyde production in neutrophils [61,62]. In addition, a stimulatory role for LTB_4 in IL-1, IL-2 and IL-6 synthesis has been suggested [62]. Moreover, LTB_4 has been shown to bind peroxysomal proliferator activated receptor (PPAR)α [63]. On the other hand, Cys-LTs are eosinophil chemoattractants, cause plasma leakage from post-capillary venules, enhance mucus secretion and induce synthesis and release of pro-inflammatory mediators including IL-8 and platelet activating factor [6,64]. The involvement of LTs in the pathogenesis of inflammatory disorders has been firmly established over the last two decades. Asthma and allergic rhinitis represent a paradigm of exacerbated 5-LO activity. In fact, inhibition of the 5-LO pathway in these conditions exerts clinically relevant benefitial effects [65,66]. The 5-LO pathway is also emerging as a therapeutic target in ostheoarthritis, in which increased LTB_4 formation appears to be pathogenetically relevant [67]. Furthermore, 5-LO has been identified as a major gene involved in atherosclerosis susceptibility in mice [68]. Components of the 5-LO pathway including 5-LO, FLAP, LTs and their receptors, LTA_4 hydrolase and LTC_4 synthase are overexpressed in aortic, coronary and carotid atherosclerosis lesions in humans [69].

Leukotriene-Modifying Drugs

Over the past 20 years, a number of pharmacological agents that modify the 5-LO metabolic pathway and the biosynthesis of LTs have been developed to treat inflammatory diseases such as asthma, ulcerative colitis, arthritis and psoriasis. These agents, which are generically known as leukotriene-modifying drugs, include 5-LO inhibitors, FLAP inhibitors and Cys-LT receptor antagonists. 5-LO inhibitors that directly block the enzyme´s activity were the first pharmacological compounds considered as leukotriene-modifying drugs. Many of the molecules originally developed were discarded because of severe side effects and were never marketed, although some are currently used for *in vitro* research [reviewed in reference 70]. Nordihydroguaretic acid (NDGA), caffeic acid, AA-861 and BW-775C fall within this category. Other compounds such as the non-redox inhibitor ZD-2138, showed good *in vitro* activity, but failed to give substantial response *in vivo*. Although a series of imidazole compounds derived from ZD-2138 showed low IC_{50} and good *in vivo* activity following oral administration, CJ-12918, the most active metabolite among these molecules, was discontinued because of toxicity [71]. Molecules designed to chelate the active iron were also developed as 5-LO inhibitors. One of these, containing a N-hydroxyurea derivative and denominated zileuton, efficiently inhibits 5-LO with an IC_{50} of 0.5-1μM [72]. Zileuton (*Zyflo*®) is currently marketed as an anti-asthmatic drug. To improve potency and half-life after oral administration, other molecules such as ABT-080, a symmetrical bis(quinolylmethoxyphenyl) alkylcarboxylic acid derivative with an IC_{50} of 16-20 nM and a half life of ~ 6-9 h, have been synthesized [73]. A different approach to inhibit the 5-LO pathway is by means of FLAP inhibitors. Several compounds that interact with FLAP such as MK-886, BAY-X-1005 and MK-0591 have been designed and their properties and potency characterized [reviewed in references 74-76]. FLAP inhibiton has recently attracted much attention in cardiovascular inflammation because linkage analysis has demonstrated that the gene encoding for FLAP confers a higher risk of myocardial infarction and stroke [77]. Receptor antagonists are an important new class of leukotriene-modifying drugs. Orally active receptor antagonists directed against the Cys-LT_1 receptor have been marketed in the last few years [74-76]. The Cys-LT_1 receptor antagonists montelukast (*Singulair*®), pranlukast (*Ultair*®) and zafirlukast (*Accolate*®) have been tested in a number of clinical trials and have demonstrated that they improve pulmonary function and reduce asthma exacerbations, especially in exercise-induced asthma [78]. On the other hand, LTB_4 receptor antagonists (i.e. SC-41930 or CP-105.696) have been shown to be efficacious in reducing the arthritis index and ankle bone destruction in IL-1-accelerated collagen-induced arthritis and in reducing atherosclerosis lesion progression in mice [79,80].

CYTOKINES AND GROWTH FACTORS

Cytokines and growth factors are low-molecular-weight mediators of cellular communication [81]. Almost every cell in our organism, including most types of liver cells, has the ability to produce and secrete cytokines. Once released, cytokines interact with specific receptors in their target cells where they induce multiple responses in both an autocrine and paracrine fashion (i.e. interacting with the same or neighboring cells). Moreover, most cytokines are pleiotropic and induce multiple overlapping effects [81]. The most important cytokines are interleukins (IL) (there are currently 18 different interleukins), tumor necrosis factor (TNF), interferon (IFN) and tumor growth factor (TGF). These cytokines can be classified according to their role in infection and/or inflammation. The prototypical proinflammatory cytokines are IL-1 and TNF-α, whereas IL-4, IL-10 and IL-13 are considered anti-inflammatory cytokines [81]. There are certain cytokines that are specifically involved in the regulation of immune response and are known by the term chemokines (i.e. IL-8). Among the different cytokines, and because of their role in liver inflammation, matrix remodelling and fibrogenesis, in the current review we will focus our discussion on TNF-α and TGF-β.

TNF-α

TNF-α is one of the most important proinflammatory cytokines. Although this cytokine exerts pleiotropic effects in the context of the liver, its major effects are associated with the regulation of hepatocyte proliferation and apoptosis and the induction of inflammatory response [82,83]. The biological effects of TNF-α are mediated by its interaction with two plasma membrane receptors: TNF-R1 and TNF-R2.

In most cells, binding to TNF-R1 induces apoptosis, although a role for TNF-R2 in T cell-mediated hepatocyte apoptosis can not be discarded [84]. TNF-α responses also appear to be mediated by the transcription factor NF-κB [85]. In most circumstances, TNF-α activates the release of a number of cytokines such as IL-1 and IL-6 by liver macrophages which cause hepatic injury and fibrosis [86]. It has become clear that TNF-α is implicated in the progression of liver disease. In this regard, TNF-α has been shown to be a critical regulator of hepatocyte physiology in fulminant hepatic liver failure [87], liver allograft rejection [88], chronic hepatitis B virus infection [89-91] or alcoholic hepatitis [92]. In addition, TNF-α together with INF-γ play a major role in the pathogenesis of autoimmune liver disease and cholestasis [93,94]. Moreover, human studies have shown a genetic association between TNF-α promoter polymorphisms and the risk of developing hepatocellular carcinoma [95], liver allograft rejection [96], advanced alcoholic liver disease [97] or fulminant hepatitis [98]. The role of TNF-α in liver injury has also been extensively studied in several animal models of liver disease. For example, TNF-α appears to be involved in carbon tetrachloride (CCl_4)-induced liver damage since TNFR1/TNFR2-deficient mice are resistant to the development of histological fibrosis after 8-weeks of CCl_4 treatment [99]. Although, TNF-α is a key target gene in most of these models, there are significant differences in its mechanism of action. For example, in the galactosamine (D-(+)-GalN) model, a hepatotoxin that selectively blocks transcription in hepatocytes by depleting uridine nucleotides [100], TNF-α induces activation of caspases and subsequent hepatocyte apoptosis, infiltration of leukocytes and macrophages, finally leading to death [101,102]. TNFR1 plays an essential role in this experimental model, since TNFR1 deficient mice are resistant to develop liver injury [103]. In contrast, TNF-α appears to be necessary for liver regeneration following radical hepatectomy [104]. Because of its key role in the progression of liver disease, potential anti-TNF-α therapies are of interest. One therapeutic drug available is infliximab (*Remicade®)*, a chimeric monoclonal antibody cA2 against TNF-α. Infliximab is currently used in the treatment of severe active Crohn's disease [105] and rheumatoid arthritis [106]. In these diseases, infliximab is effective and well-tolerated, and has become a widely used treatment. Moreover, the results of clinical trials, open-labelled studies, and case studies indicate that TNF-*α* inhibitors look very promising for the treatment of a variety of other conditions including uveitis, sarcoidosis, Sjögren's syndrome, Behçet's syndrome, vasculitis, and graft versus host disease [107]. There is also a rationale for using TNF-α blockade in systemic lupus erythematosus, a prototype of autoimmune-mediated disease. Regarding the liver, there are two preliminary trials reporting beneficial effects of infliximab in patients with severe alcoholic hepatitis [108,109]. However, severe adverse effects (i.e. an increased incidence of severe infections) were subsequently reported in patients receiving infliximab and the clinical trial was stopped by the French Drug Agency [110]. Therefore, there are serious concerns about the safety of this therapy in liver disease, especially after detecting episodes of chronic hepatitis B reactivation [111-113] or autoimmune hepatitis [114] in patients with Crohn's disease or psoriatic arthritis [111-114] treated with infliximab.

TGF-β

TGF-β is another key mediator involved in the progression of liver disease. TGF-β plays a major role in the pathogenesis of liver fibrosis by activating hepatic stellate cells (HSCs) (Ito cells, fat-storing cells), the major cellular type involved in liver fibrogenesis [115]. Specifically, in response to TGF-β, HSCs undergo phenotypic changes, switching from a quiescent vitamin A-rich phenotype to a myofibroblastic phenotype. Activated HSCs produce most of the extracellular matrix components leading to liver fibrosis and also secrete many proinflammatory cytokines and chemokines, such as TNF-α and TGF-β itself, which, in turn, promote activation of neighboring quiescent HSCs. The critical role of TGF-β in this condition is supported by experimental studies showing that overexpression of TGF-β in transgenic mice induces spontaneous liver fibrosis [116] whereas disruption of TGF-β synthesis and/or signalling pathways significantly decreases hepatic matrix deposition [117]. In humans, increased production of TGF-β has been observed in livers from patients with alcoholic cirrhosis [118].

OXIDATIVE STRESS

Biological processes such as energy generation by mitochondria and detoxification reactions inevitably generate free radicals, unstable molecules with an unpaired electron that makes them highly reactive [119]. There are also exogenous conditions that can enhance the production of free radicals in our organism, including cigarette smoking, alcohol consumption and ionizing radiations [120]. The most common free radicals are products of oxygen and nitrogen metabolism that are known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively (Table **1**). In addition, free radicals can combine to form other more damaging or toxic species such as peroxynitrite ($ONOO^-$), a product of superoxyde and nitric oxide radical reaction. In general, the term ROS is used to refer to both radical (i.e. superoxide) and non-radical (lipid peroxide) reactive-oxygen derivatives. ROS readily react with organic substrates such as lipids, proteins and DNA, damaging our cells and tissues and promoting the development of several diseases [121]. Luckily, a number of antioxidant systems such as superoxide dismutase and glutathione peroxidase are present in our organism to quench ROS. In healthy subjects, the quenching is seamless and balanced. Under certain circumstances, however, ROS could be over-produced or the quenching can become insufficient, leaving a net excess amount of ROS in the system [122]. Such abnormal levels of ROS then cause oxidative stress, which leads to inflammatory consequences. In fact, there is a close relationship between oxidative stress and inflammation. Oxidative stress is believed to be involved in the progression of liver disease. An overproduction of free radicals and an increase in hepatic lipoperoxidation have been reported in alcoholic liver disease, liver cirrhosis and steatohepatitis [119,123,124]. At the experimental level, there are many studies supporting a central role for oxidative stress in the pathogenesis and progression of liver disease. In CCl_4-induced liver damage, a useful animal model, in which hepatic lipid peroxidation is produced by the generation of $Cl_3^{\bullet}$ radicals by the cytochrome P450 system, the administration of antioxidants as drug coadjuvants, efficiently counteract liver damage. Specifically, dietary supplementation with

Table 1. List of the Most Relevant Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

ROS	
Radicals	
Hydroxyl	$OH^{\bullet}$
Superoxide	$O_2^{\bullet -}$
Nitric oxide	$NO^{\bullet}$
Thyol	$RS^{\bullet}$
Peroxyl	$RO_2^{\bullet}$
Lipid peroxyl	$LOO^{\bullet}$
Non-Radicals	
Peroxynitrite	$ONOO^-$
Hypochloric acid	HOCl
Hydrogen peroxide	H_2O_2
Singlet oxygen	$^1\Delta_g(^1O_2)$
Ozone	O_3
Lipid peroxide	LOOH
RNS	
Nitrous oxide	$NO^{\bullet}$
Peroxynitrite	$OONO^-$
Peroxynitrous acid	ONOOH
Nitroxyl anion	NO^-
Nitrosyl cation	NO^+
Nitrogen dioxide	$NO_2^{\bullet}$
Dinitrogen trioxide	N_2O_3
Nitrous acid	HNO_2

zinc, vitamin E as well as S-adenosyl-methionine (SAME) has been shown to ameliorate liver injury in this toxic model of liver disease [125-130].

KUPFFER CELLS ARE KEY TARGETS FOR THE MODULATION OF LIVER INFLAMMATION AND OXIDATIVE STRESS

Kupffer cells are liver macrophages that lie in the hepatic sinusoids in direct contact with the blood. Kupffer cells represent the largest population of resident macrophages in the body. Under physiological conditions, the main functions of Kupffer cells include the phagocytic elimination of exogenous particles as well as endotoxin detoxification [131]. However, under certain pathological circumstances, for example in chronic alcoholic hepatitis, Kupffer cells have enhanced sensitivity to endotoxin and become activated [132]. Once activated, Kupffer cells release a number of biologically active compounds including oxygen derived free radicals, nitric oxide, proteases, cytokines and growth factors (i.e. TNF-α, TGF-β, IL-1 and IL-6) and especially proinflammatory lipid mediators derived from arachidonic acid (eicosanoids) (Table **2**). The massive release of these molecules, along with the release of lysosomal enzymes induces hepatocyte necrosis and liver inflammation. Since activation of Kupffer cells and the subsequent release of potent proinflammatory mediators is considered to be an early step in the pathogenesis of liver damage and tissue remodeling, modulation of these cells provides a potential therapeutic strategy in liver disease. In fact, the number of macrophages is consistently increased and closely correlates with the degree of hepatic injury in experimental models of liver disease [133-136]. In these models, the degree of steatosis, inflammation, necrosis and collagen content is significantly attenuated by depletion of Kupffer cells by treatment with gadolinium chloride [137-140]. Similar findings have been observed in CD11b-DTR transgenic mice, which are selectively depleted of Kupffer cells [141]. In our laboratory, we have been able to selectively deplete Kupffer cells by means of an agent that blocks the binding of 5-LO to its accessory protein, FLAP. The 5-LO pathway is essential for cell survival [142] and we and others have demonstrated that the inhibition of this pathway stops growth-related signals and induces programmed cell death in cells in culture [143]. Since expression of 5-LO in the liver is basically restricted to macrophages [143,144], 5-LO inhibition provides a selective strategy for depleting Kupffer cells. Indeed, the administration of Bay-X-1005 (a potent and selective FLAP inhibitor) to rats with CCl_4-induced damage produced a partial depletion of Kupffer cells and was associated with a remarkable hepatoprotective action in terms of reducing necroinflammatory liver injury [145]. In addition to their role in liver inflammation, Kupffer cells are also recognized for their activity in promoting the activation of HSCs through release of paracrine factors [1-3,86,146,147]. Therefore, the activation of Kupffer cells is considered to be a critical event in the initiation of the inflammatory cascade leading to liver fibrosis in CCl_4-treated rats [86,133,143]. For this reason, inhibition of the 5-LO pathway with Bay-X-1005 has also been associated with significant anti-fibrotic effects [143]. In these animals, Bay-X-1005 also induced a reduction in the gelatinolytic activity of matrix metalloproteinase-2 (MMP-2) and a decrease in mRNA expression of tissue inhibitor of MMP-2 (TIMP-2) [145].

On the other hand, inhibition of COX in Kupffer cells is also of interest to control the inflammatory process in the liver. Since inflammation is histologically present in virtually all forms of liver disease, several anti-inflammatory strategies have been tested in this disease. Glucocorticoids and colchicine, for example, are representative anti-inflammatory agents of potential therapeutic use in alcoholic hepatitis, although the efficacy of these drugs remains of unproven value as anti-fibrotic agents [148]. Although, NSAIDs appear to be useful in preventing the development of experimental liver fibrosis, cirrhosis and hepatic focal lesions caused by a choline-deficient diet [149], unfortunately, these drugs are not recommended in patients with liver disease because of renal side effects [42]. Since selective COX-2 inhibitors have been shown to spare the renal function in cirrhosis [43,44,150], these compounds represent a novel therapeutic strategy to dampen liver inflammation. In our laboratory, we have been able to prevent liver fibrogenesis by administering SC-236, a selective COX-2 inhibitor, to CCl_4-treated rats [151]. The mechanisms by which SC-236 exerts

Table 2. List of Biologically Active Compounds Derived from Kupffer Cells

Cytokines	IL-1, IL-6, IL-10, TNF-α, IFN-α, IFN-γ, TGF-β, MIP-1, MCP-1.
Complement Cascade	C5a
Eicosanoids and Platelet Activator Factor (PAF)	PGI_2, PGE_2, PGD_2, PGF_2, TXA_2, LTB_4, cysteinyl-LT, 15-epi-LXA_4.
Extracellular Matrix Components	Fibronectin, Proteoglicans
Lysosomal Enzymes	Catepsin, β-glucoronidase, β-acetyl glucosaminase, Peroxidase, Esterases, Acetylases.
Nitrogen Reactive Species	Nitric oxide, Nitrate, Nitrite.
Oxygen Reactive Species	Superoxide anion, Hydrogen Peroxide, Hydroxyl Radical.
Proteases and Lysozyme	Plasminogen activator, MMP-9, MMP-13, MMP-2, MMP-14.

IL: interleukin; INF: interferon; TGF: transforming growth factor; MIP-1: macrophage inflammatory protein-1; MCP-1: monocyte chemoattractant protein-1; PG: prostaglandin; TXA_2: thromboxane A_2; LT: leukotriene; LX: lipoxin ; MMP: matrix metalloprotease.

antifibrotic effects are not well defined, but include the inhibiton of proinflammatory PGs, the induction of apoptosis in Kupffer cells and HSCs as well as COX-inde-pendent pathways such as activation of PPARγ [143,150]. PPARγ is a ligand-activated transcription factor with a DNA binding domain that recognizes response elements in the promoter region of specific target genes linked to inflammation, cell proliferation, apoptosis and differentiation [152-155]. PPARγ plays a pivotal role in the progression of liver fibrosis since HSC activation is associated with a reduction in both expression and transcriptional activity of this nuclear receptor [156,157]. In our study, the selective COX-2 inhibitor, SC-236, increased PPARγ expression in HSCs and restored its expression to normal levels in the liver of CCl_4-treated rats [151]. Furthermore, SC-236 worked as a direct ligand of PPARγ in a transactivation assay [151,152]. This finding is important since the administration of synthetic PPARγ ligands (i.e. antidiabetic thiazolidinediones) to animal models of liver fibrosis effectively reduces HSC transdifferentiation and collagen deposition [158]. Moreover, human studies have shown a beneficial effect of thiazolidinedione rosiglitazone by reducing hepatic steatosis and fibrosis in patients with non-alcoholic steatohepatitis (NASH) [159].

CONCLUDING REMARKS

In conclusion, several evidence indicate that COX-2 and 5-LO pathways are involved in the pathogenesis of liver inflammation and fibrosis. In addition, the participation of proinflammatory and profibrogenic cytokines such as TNF-α and TGF-β as well as oxidative stress in liver disease has been firmly established. Consequently, modulation of the eicosanoid cascade and the biological effects of TNF-α or TGF-β and the balancing of oxidative stress status are potential targets for design and discovery of new drugs to reduce liver inflammation and injury.

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