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PPAR α in atherosclerosis and inflammation

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Summary

Peroxisome proliferators-activated receptor (PPAR) α is a nuclear receptor activated by natural ligands such as fatty acids as well as by synthetic ligands such as fibrates currently used to treat dyslipidemia. PPAR α regulates the expression of genes encoding proteins that are involved in lipid metabolism, fatty acid oxidation and glucose homeostasis, thereby improving markers for atherosclerosis and insulin resistance. In addition, PPAR α exerts anti-inflammatory effects both in the vascular wall and the liver. Here we provide an overview of the mechanisms through which PPAR α affects the initiation and progression of atherosclerosis, with emphasis on the modulation of atherosclerosis-associated inflammatory responses. PPAR α activation interferes with early steps in angiogenesis by reducing leukocyte adhesion to activated endothelial cells of the arterial vessel wall and inhibiting subsequent transendothelial leukocyte migration. In later stages of atherosclerosis, evidence suggests activation of PPAR α inhibits the formation of macrophage foam cells by regulating expression of genes involved in reverse cholesterol transport, formation of reactive oxygen species (ROS), and associated lipoprotein oxidative modification among others. Furthermore, PPAR α may increase the stability of atherosclerotic plaques and limit plaque thrombogenicity. These various effects may be linked to the generation of PPAR α ligands by endogenous mechanisms of lipoprotein metabolism. In spite of this dataset, other reports implicate PPAR α in responses such as hypertension and diabetic cardiomyopathy. Although some clinical trials data with fibrates suggest that fibrates may decrease cardiovascular events, other studies have been less clear, at least in the presence of concomitant statin therapy. Independent of the clinical effects of currently used purported PPAR α agonists, extensive data establishes the importance of PPAR α in the transcriptional regulation of lipid metabolism, atherosclerosis and inflammation.

Introduction

Atherosclerosis, the major cause of death from cardiovascular disease in industrialized countries, is characterized by the progressive accumulation of lipid and fibrous depositions in the vessel wall of large arteries [1,2]. Well-established risk factors for atherosclerosis include hypertension, hypercholesterolemia, and diabetes mellitus. More recent work reveals procoagulant and proinflammatory states can be added as important contributors to the development of atherosclerosis [3–5]. To an increasing extent, attention has focused on how abnormalities of metabolism – atherogenic dyslipidemia, insulin resistance, visceral adiposity – may promote atherogenesis. Indeed, clinical evidence underscores how parameters such as glucose represent a continuous rather than dichotomous variable in cardiovascular risk, even at levels that do not meet a diagnosis of frank diabetes. Likewise, although debates continue regarding triglycerides as an independent risk factor for cardiovascular events, the presence of

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hypertriglycemia confers a considerable increase in risk among subjects with otherwise similar ratios of low density lipoprotein (LDL) and high density lipoprotein (HDL).

This evolving view of atherosclerosis as a metabolic complication has directed attention towards peroxisome proliferators-activated receptors (PPARs) as transcriptional regulators involved in lipid metabolism, inflammation and atherosclerosis. PPARs, as ligand-activated transcription factors belonging to the nuclear hormone receptor family, can regulate multiple target genes. Extensive data establishes expression of all three PPAR isotypes – PPAR α , - γ , and - δ/β – throughout the vasculature and inflammatory cells [8]. The focus here is on PPAR α , which has been strongly implicated in beta oxidation of fatty acids as well as lipid metabolism. Not surprisingly given these effects, PPAR α is expressed mainly in higher energy-requiring tissues like skeletal muscle and heart as well as the liver. Activation of PPAR α has been reported to improve levels of triglycerides, HDL, and the overall atherogenic plasma lipid profile, while also potentially modulating inflammation as well insulin resistance itself [6,7]. PPAR α has been reported to be activated by natural ligands such as fatty acids and their derivatives, and lipoprotein lipolytic products [9,10], as well as by drugs such as the lipid-lowering fibrates [11–13]. Pharmacological treatment of patients with fibrates has been shown to lower cardiovascular mortality although this data set is mixed as will be discussed further below.

One of the many lines of evidence that suggest PPAR α may play a role in atherosclerosis derives from the data implicating this receptor in limiting inflammation. In the absence of PPAR α , mice have a prolonged response to inflammatory stimuli [9]. PPARs have also been found to modulate the acute phase response of the liver as well as mechanisms of inflammation in the vasculature [14,15]. Aortas from PPAR α -deficient mice display an exacerbated inflammatory response to stimulation with lipopolysaccharide. In addition, murine endothelial cells (EC) and hepatocytes that lack PPAR α have increased levels of inflammatory targets such as vascular cell adhesion molecule-1 (VCAM-1) and serum amyloid A (SAA) [10,16].

PPAR α 's seeming placement at the nexus of lipid metabolism, energy balance and inflammation makes its potential as a target for limiting atherosclerosis of obvious interest. This review examines the various mechanisms through which PPAR α has been implicated in atherosclerosis and explores the potential importance of PPAR α in atherosclerosis. Although inflammation is an important part of the immune response and necessary for organismal defense, chronic inflammatory activation may also have deleterious, maladaptive effects, including promotion of atherosclerosis and its complications. Given the evidence for PPAR α 's involvement in limiting inflammation under basal conditions and inhibiting inflammatory responses after inflammatory stimuli forces, this issue will be a focus of the discussion here.

PPAR α in the regulation of inflammation in the liver

The liver is an integral and often overlooked player in atherosclerosis, including systemic effects through hepatic function as the organ seat for the synthesis of lipoprotein particles – important contributors to cardiovascular risk. The liver is also the site of synthesis for other acute phase reactants such as C-reactive protein (CRP), fibrinogen and serum amyloid A (SAA). Levels of these proteins have all been shown to correlate with cardiovascular disease [17,18]. The acute phase response is a generalized reaction of an organism to injury and consists of the increased or reduced secretion of a large number of acute phase proteins by the liver in response to injury and various stressors, including cytokines secreted by neutrophil granulocytes and macrophages. Fibrinogen is directly involved in promoting thrombosis while recent evidence also hypothesizes potential direct roles for SAA and CRP in atherosclerosis as well. These proteins are all induced by the inflammatory cytokines interleukin (IL)-6, IL-1

and tumour necrosis factor (TNF) α (Figure 1) [16,19,20]. Interestingly, the levels of these inflammatory cytokines also correlate with obesity, suggesting one mechanism that may link obesity and atherosclerosis [21].

PPAR α agonists decrease IL-1-induced CRP expression in primary human hepatocytes and induction of plasma CRP levels by IL-1 in human CRP-expressing transgenic mice [22,23]. In these human CRP transgenic mice, PPAR α activation also reduced basal plasma CRP levels, i.e. in the absence of an inflammatory stimulus. At the transcriptional level, the reduction in CRP levels may derive from an increase in I κ B α protein upon PPAR α activation. Binding of I κ B α to the p50 subunit of nuclear factor (NF) κ B reduces translocation of this key proinflammatory transcription factor to the nucleus, resulting in decreased transcription of multiple inflammatory response genes. p50-NF κ B complexes with CCAAT/enhancer binding protein (C/EBP) α in the nucleus to drive expression of these targets, including CRP [23]. In addition, PPAR α activation in mouse liver also reduces CRP expression by decreasing the protein levels of C/EBP α and NF κ B themselves [22]. In humans, PPAR α agonists can also repress levels of CRP, a topic of intense interest in the field of cardiovascular risk biomarkers. Similar effects of PPAR α activation have also been reported on fibrinogen and SAA [15,24–26]. In mice that lack PPAR α , the basal expression of SAA in primary hepatocytes is markedly increased while in wild-type mice, PPAR α agonist treatment represses the increase in SAA expression in response to stimulation with inflammatory cytokines [16,26]. PPAR α agonists also reduce plasma concentrations of TNF α , interferon (IFN)- γ , IL-6 and IL-1 induced expression of IL-6 [15,27]. Repression of each of these cytokines would be expected to have distal effects on numerous vascular and other inflammatory cells responses. In addition to the NF κ B complex and C/EBPs, the inhibitory action of PPAR α on cytokine expression includes interference with other transcription factors such as the activator protein-1 (AP-1) complex, including c-fos and c-jun, and signal transducer and activator of transcription proteins (STAT) [14,22,26,28].

Taken together, this data for PPAR α limiting hepatic cytokine expression involving multiple targets and various transcriptional effects suggests a primordial role for PPAR α in limiting inflammatory responses in the liver. Importantly, these responses are not limited to hepatic responses to synthetic PPAR α agonists, but also appear to extend to endogenous mechanisms of PPAR α activation. Under basal conditions in the genetic absence of PPAR α , the hepatic inflammatory target gene serum amyloid A is significantly induced absent any other stimulus [16]. Such effects further implicate PPAR α as a tonic repressor of inflammation. Systemically, these PPAR α effects would be expected to result in potent modulation of the acute phase response reaction as well as reduced expression of hepatic proteins implicated as markers and/or mediators of cardiovascular risk, including broader effects on inflammation itself. Certainly these changes in hepatic responses and circulating proteins can have systemic effects, including on the arterial wall. Beyond this, PPAR α may also directly modulate responses in the vasculature as discussed further below.

PPAR α and endothelial reactivity

Development of atherosclerotic lesions is often preceded by abnormalities in vascular wall reactivity [29]. In many ways, this alteration in arterial function stems from changes in endothelial cells, highlighting one of many examples that have re-defined the endothelium as a dynamic, biologically-active organ rather than just a passive arterial lining. The importance of the endothelium in vessel reactivity and subsequent abnormalities of arterial responses has fostered the use of the term “endothelial dysfunction”. Importantly, endothelial dysfunction includes alterations in any of the functional roles the endothelium plays *in vivo*, including maintenance of normal tone, limiting thrombosis, and protecting against leukocyte adhesion. Reactivity of the arterial wall is controlled in part by biomechanical inputs, including blood

flow and blood pressure. Vessel wall tone is a carefully controlled parameter regulated in part by ECs and their production and regulation of opposing signals. Endothelin-1 (ET-1) promotes blood vessel constriction, as evident with the decrease in blood flow after endothelial ET-1 release. ET-1 also induces smooth muscle cells (SMCs) proliferation. These ET-1 responses are countered by endothelial release of nitric oxide (NO), which stimulates vasodilatation, thus increasing blood flow. Endothelial nitric oxide synthase (eNOS) is the key enzymatic step in producing NO, a secondary messenger produced by EC that can inhibit NF κ B activation and attenuate endothelial inflammatory responses, including adhesion molecule expression. Current models suggest that cardiovascular risk factors, including mechanical forces like hypertension and shear stress, shift the balance between these opposing forces, increasing ET-1 production and decreasing NO production, resulting in increased vasoconstriction, abnormal vasomotor responses and promotion of atherosclerosis.

PPAR α has effects on both these countering endothelial limbs involving ET-1 and NO. By inhibiting the AP-1 signaling pathway noted earlier, PPAR α activation has been reported to inhibit thrombin-mediated induction of ET-1 [30]. In addition, in EC, the induction of ET-1 release by oxidized low-density lipoprotein (ox-LDL) is inhibited by PPAR α agonists [31]. In terms of NO, PPAR α agonists reportedly enhance eNOS expression and NO release although data in this regard has been variable [32,33]. Excessive NO production may enhance formation of peroxynitrite, possibly promoting oxidative stress [34]. PPAR α involvement in other NO pathways has also been suggested, including inhibition of inducible nitric oxide synthase (iNOS) expression by murine macrophages [35]. Together, the effects on ET-1 expression and NO release suggest that PPAR α activation in the endothelium may confer a vasoprotective effect (Figure 2) in the endothelium, limiting one component of endothelial dysfunction. These responses may contribute to improvements in vascular reactivity reported in hypertriglyceridemic human subjects in response to fibrate treatment [36].

PPAR α and the arterial adhesion and entry of leukocytes

Early atherogenesis is characterized by the recruitment and subsequent entry of leukocytes to an injured endothelium. This endothelial damage can derive directly from risk factors such as hypertension (shear stress), hyperglycemia and hypercholesterolemia. Leukocytes are recruited to these sites of injury by following a chemical gradient of released chemoattractant cytokines, or chemokines, released from the activated endothelial cells and the sub-endothelium. Subsequently, these inflammatory cells undergo a discrete series of steps that allow their attachment to the endothelial monolayer at the site of injury, a process mediated by adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1, Figure 2), intercellular adhesion molecule (ICAM)-1 and E-selectin. Ultimately, these adhered leukocytes can trans-migrate into the intima, the innermost layer of the vascular wall [37]. The expression of VCAM-1, which binds predominantly monocytes and T lymphocytes (Figure 3 and Figure 4), is induced by proinflammatory cytokines such as TNF α , IL-1 and IL-6. VCAM-1 deficient mice bred on to an atherosclerosis susceptible LDL-receptor deficient background exhibit reduced lesion formation [38]. Interestingly, an atherogenic diet induces VCAM-1 expression in endothelial cells in mice while certain omega 3 fatty acids repress VCAM-1 induction [39,40]. Importantly, leukocyte adhesion is a critical element in host defenses, allowing inflammatory cells to be marshalled to sites of injury and inflammation, for example during infections. In terms of atherosclerosis, these responses may be maladaptive, potentially leading to the formation of complex plaque prone to rupture. Interestingly, circulating levels of soluble forms of adhesion molecules like VCAM-1 correlate with cardiovascular risk.

Considerable evidence suggests PPAR α may limit endothelial responses that promote leukocyte adhesion and entry into the vessel wall. PPAR α agonists inhibit transcriptional expression of VCAM-1 induced by inflammatory cytokines such as TNF α , as evident in terms

of mRNA, protein and VCAM-1 promoter responses, with the latter deriving at least in part from NF κ B inhibition [41,42]. The repression of VCAM-1 expression by PPAR α agonists does not occur in ECs from PPAR α -deficient mice, providing a genetic argument that the effects of these drugs are indeed through PPAR α and a pharmacologic 'off-target' response. Moreover, basal levels of VCAM-1 in microvascular endothelial cells from PPAR α deficient mice are increased as compared to EC isolated from wild-type mice absent any other intervention, implicating PPAR α as a tonic inhibitor of inflammation. Endogenous pathways of lipid metabolism, like hydrolysis of VLDL by lipoprotein lipase or HDL hydrolysis by endothelial lipase, that activate PPAR α can also limit cytokine-induced VCAM-1 expression [10,94].

The data for PPAR α activation inhibiting expression of ICAM-1 and chemokines are less clear than for PPAR α 's effects on VCAM-1. In humans, treatment with fenofibrate reduced ICAM-1 plasma levels, however, the observed effect might have been the result of modified lipid levels rather than a direct effect of PPAR α agonists [43,44]. In addition, in contrast to VCAM-1 deficient mice, atherosclerotic lesions in the aorta of mice deficient in both ICAM-1 and the LDL-receptor were not reduced in response to PPAR α agonist treatment [38]. In terms of chemokines, while PPAR γ agonists clearly repress expression of subsets of IFN- γ inducible chemokines, PPAR α agonists do not appear to have similar effects, at least in human ECs [45]. Certain oxidized phospholipids have been reported to activate PPAR α , resulting in increased expression of MCP-1 and IL-8 in ECs, one of a few reports that suggest possible proinflammatory effects of PPAR α in ECs [46]. CRP-induced MCP-1 expression in human umbilical vein ECs (HUVEC) has been reported to be inhibited by synthetic PPAR α activators [47]. Multiple factors may help explain these somewhat variable PPAR α responses in EC, including experimental issues such as the specific nature of the ECs, the agonists employed, concentrations used, as well as distinct biologic effects that may depend on cell type and species. In general, PPAR α activation seems to inhibit early processes contributing to atherosclerotic plaque formation. Whether clinical responses to putative drugs that are thought to target PPAR α support this body of data is discussed further below.

PPAR α and local immune cell responses

T lymphocytes and dendritic cells (DCs) are now recognized as important players in atherosclerosis, with the recruitment, activation and proliferation of these immune cells contributing to lesion formation and its complications. Activated T lymphocytes and DCs, the most potent antigen-presenting cells, co-localize in atheromata [48]. After their chemokine recruitment and entry into the vessel wall, T lymphocytes, consisting of mainly CD4-positive cells, differentiate from naive Th0 cells into Th1 cells after stimulation by antigens such as oxidized LDL [49]. Th1 cells secrete predominantly pro-inflammatory cytokines such as IFN- γ , TNF α , and IL-2, which can then activate other cellular participants in atherosclerosis (Figure 3). For example, IFN- γ stimulates cytotoxic T lymphocytes and monocyte/macrophages. The promotion of atherogenesis through Th1 cell-mediated cytokine production is exemplified by the reduction in atherosclerotic lesions in mice with decreased IFN- γ activity [50]. Importantly, smooth muscle cells (SMC) also being activated by similar cytokine mechanisms, promoting SMC activation [14,15].

The potential impact of PPAR α activation on these inflammatory pathways may derive from effects on proximal steps in the amplification cascade of the immune signaling summarized above. PPAR α activation reduces the T cell production of both IFN- γ and IL-2, thus possibly modulating multiple distal responses to these cytokines [51,52]. In SMCs, PPAR α inhibits IL-1 β induced expression of cyclooxygenase (COX)-2 via interference with the NF κ B and AP-1 pathways [15]. At sites of inflammation, COX2 promotes increased production of various inflammatory mediators (Figure 3). Expression of COX2 is increased in atheroma. Overall,

PPAR α appears to reduce proinflammatory cytokine production by T cells, effects that would be expected to limit vascular inflammatory responses and atherosclerosis.

PPAR α on oxidative modification of lipoproteins and foam cell formation

Elevated LDL-cholesterol is a well-established risk factor for cardiovascular disease (CAD), as evident in the strong association between genetic disorders of cholesterol metabolism characterized by marked elevated LDL levels and premature CAD; patients with familial hypercholesterolemia can have myocardial infarctions as early as 1 to 2 years of age. In more common forms of CAD, LDL confers an increased risk of atherosclerosis across a very wide range of LDL levels. Indeed, as clinical trial experience with LDL-lowering therapies has grown, the 'desirable' target levels of LDL have fallen. Many patients with CAD have LDL and total cholesterol levels that fall well within the lower ranges of average LDL and cholesterol levels in a given national population. However LDL and total cholesterol values vary widely throughout the world, with a direct correlation between these levels throughout the world and cardiovascular disease mortality. Hence, it is difficult to reference any given LDL value as being "normal". Indeed, patients with diabetes often have average to low LDL levels despite their obvious atherosclerosis risk [53]. This LDL may be smaller and denser, properties linked to retention in the arterial wall, increased susceptibility to oxidation and increased cardiovascular risk [54–56]. Once in the arterial wall, LDL is thought to undergo oxidation and uptake by infiltrating monocytes in the subendothelial intimal layer, promoting the differentiation of these monocytes into macrophages and ultimately foam cells [57]. Foam cells also produce cytokines and growth factors, further promoting atherosclerosis and are also postulated to serve as a source of matrix metalloproteinases, enzymes implicated in the weakening and rupture of the fibrous cap, the process thought to underlie most myocardial infarctions (Figure 4).

PPAR α -activating fibrates increase LDL size and reduce LDL density in dyslipidemic patients [58–60]. Such changes are typically seen with triglyceride lowering and are consistent with PPAR α -mediated induction of lipoprotein lipase (LPL), a central player in triglyceride metabolism. PPAR α activation also represses expression of apoCIII, the natural inhibitor of LPL activity [61]. In addition to these potential indirect effects on LDL size and density and hence LDL oxidation, PPAR α agonists may directly modulate LDL oxidation, at least in preclinical and animal model studies. Increased PPAR α expression is associated with decreased ox-LDL in atherosclerotic plaques in insulin-resistant mice while PPAR α agonists reduce LDL susceptibility to oxidation [62,63]. Oxidative modification of LDL can also be induced by reactive oxygen species (ROS). Under basal conditions, PPAR α ligand treatment of non-activated macrophages increases ROS formation [64]. In contrast, PPAR α activation decreases the expression of the reduced form of nicotinamide dinucleotide phosphate (NADPH) oxidase, the enzyme that generates superoxide in ECs [64]. In addition, treatment with PPAR α agonist treatment increases expression of superoxide dismutases (SODs), enzymes that scavenge and process free radicals [65]. A decrease in NADPH and upregulation of SOD expression may result in decreased ox-LDL formation (Figure 4). This data suggests PPAR α may participate in determining the balance of forces involved in controlling oxidation. Interference with activation of the NF κ B pathway might be an additional mechanism through which PPAR α activation may repress superoxide mediated increases in atherogenesis [34].

In addition to modulating the size and density of LDL particles and limiting their oxidation, PPAR α may also inhibit foam cell formation by increasing reverse cholesterol transport (RCT). RCT is the process in which cholesterol is taken up from peripheral tissues by high-density lipoprotein (HDL) particles and transported to the liver for further processing and ultimate clearance. PPAR α activation increases expression of CLA-1/scavenger receptor class B type I (SR-BI) and ATP-binding cassette transporter A1 (ABCA1), genes encoding proteins

involved in apolipoprotein (apo) A-I-mediated efflux of cholesterol, an early RCT step (Figure 4). By inducing expression of these genes, PPAR α activation can increase cholesterol efflux [66,67].

In conclusion, PPAR α activation seems to limit macrophage foam cell formation, thus inhibiting the production of additional inflammatory cytokines. PPAR α activation exerts these effects by inhibiting uptake of modified LDL, limiting oxidative modification of lipoproteins and the responses to these species and by increasing expression of genes involved in reverse cholesterol transport.

PPAR α and plaque stability and thrombogenicity

During the progression from early fatty streaks to a more complex atherosclerotic lesion, SMCs proliferate, migrate, and accumulate in the atherosclerotic plaque. The production of extracellular matrix (ECM) components by SMCs can further expand the lesion and foster a more fibrous plaque. The SMC is also the source of the material that makes up the fibrous cap that separates the necrotic, lipid-rich prothrombotic core and the circulation. Rupture of the fibrous cap, which typically occurs in the shoulder region of the region where macrophages are often concentrated, exposes the pro-coagulant necrotic core of the atherosclerotic lesion to the circulating blood, activating the coagulation cascade. Thrombus formation results, which can progress to complete occlusion of the artery and subsequent distal tissue ischemia and ultimately infarction, as is seen with heart attacks and stroke. Not all plaque rupture necessarily results in total arterial occlusion. In some cases, partial occlusion of the artery can occur, manifest at times as unstable angina. Superficial erosion is also thought to be another distinct mechanism of arterial injury that can promote fibrous tissue formation and transition of fatty lesions into more fibrous ones (Figure 4).

Plaque stability can be understood as a balance of forces involving ECM formation and its degradation (figure 4). Matrix metalloproteinases (MMPs) are enzymes that can degrade ECM components such as collagen. Degradation of the ECM by MMPs is required for the SMC migration. Hence, MMPs have been strongly implicated in plaque remodeling and reduced plaque stability. Consistent with the notion of inflammation as a major contributor to atherosclerosis, both fibrous cap formation and its degradation appear to be modulated by inflammation. MMP expression in macrophages, ECs and SMCs is stimulated by inflammatory cytokines such as IL-1 and TNF α , and by ox-LDL. These vary same inflammatory pathways also decrease collagen synthesis [68,69]. Together these effects are thought to increase the potential for plaque destabilization. Apart from destabilizing plaques by secreting MMPs, monocyte-derived macrophages also contribute to thrombus formation by expressing and secreting tissue factor (TF), a potent procoagulant protein. TF has been proposed as a major contributor to the thrombogenicity of plaque.

PPAR α activation has been reported to inhibit MMP9 expression and secretion by stimulated human monocytic THP-1 cells [70]. This effect of PPAR α is indirect and may derive from superinduction of iNOS, increasing NO levels, which in turn reduce MMP9 mRNA stability [71]. Such effects may decrease the potential for plaque rupture. PPAR α activation may have other effects on plaque remodeling. PPAR α activation induces the cyclin-dependent kinase inhibitor p16, stalling SMCs at the G₁/S transition of the cell cycle and inhibiting SMC proliferation [72] (Figure 4). In addition, incubation with the fatty acid docosahexaenoic acid – a putative PPAR α activator – stimulated apoptosis of VSMCs in a p38MAPK-dependent manner [73]. The net impact of such changes in SMC biology is difficult to predict. Although such changes might limit the medial SMC proliferation common to atherosclerosis, such changes might also limit the ability of SMCs to produce the fibrous cap. This issue of how SMC biology may influence plaque stability remains generally unresolved. PPAR α activation

also enhances apoptosis of TNF α or IFN γ -activated macrophages, likely through repression of the anti-apoptotic NF κ B pathway [74].

PPAR α has also been reported to limit TF expression in human monocytes and macrophages [75,76]. PPAR α agonists inhibit activity of the TF promoter as well as TF protein levels in macrophages. It is difficult to resolve if such changes actually result in a less thrombotic lesion although this would be one potential consequence of such action on macrophages. TF is also expressed by ECs. Relatively few interventions repress TF expression. Among these, omega 3 fatty acids may do so through PPAR α activation. An additional factor implicated in determining relative thrombogenicity in plaque is the serine protease inhibitor plasminogen activator inhibitor (PAI)-1, which potently limits fibrinolysis and induces thrombosis. The balance between PAI-1 and its opposing partner tissue plasminogen activator (tPA) is thought to be especially relevant to the procoagulant picture seen in diabetes. Both increases and decreases in activity of PAI-1 have been reported after PPAR α agonist treatment, leaving the net effect unclear and providing an example of how the complexity of the PPAR system may be manifest in variable experimental responses [77–79].

In summary, PPAR α activation in SMCs and macrophages may promote the stability of atherosclerotic plaques by limiting ECM degradation, promoting macrophage apoptosis, and inhibiting SMC migration and proliferation. PPAR α agonists may also limit inflammatory cytokine production by macrophages. While repression of TF by PPAR α agonists appears plausible based on the published data, the net effect of changes in SMC as well as possible regulation of PAI-1 requires further elucidation.

Connecting lipoprotein metabolism to PPAR α activation in vivo: Implications for inflammation and atherosclerosis

Although critical early work established that certain fatty acids could bind to all PPAR isotypes, until recently, little information existed on how metabolism of lipoproteins might be connected to PPAR responses. Given the complexity of lipid metabolism, such pathways might help explain selective PPAR activation or how abnormalities in lipid metabolism might be defined in part by changes in PPAR activation. Interestingly, many aspects of the PPAR field have been defined by responses to synthetic PPAR agonists.

LPL, a member of the triacylglycerol lipase family, is an important enzyme in the hydrolysis of fatty acids from triglyceride-rich lipoproteins like VLDL. Consistent with such effects, LPL is expressed primarily in muscle, where it can help supply fatty acids for beta oxidation and energy generation, and adipose tissue, where fatty acids can be stored for future use. Although LPL is not synthesized by the endothelium, LPL encounters its substrates, such as circulating VLDL, while positioned on the endothelial surface [80,81]. Recent work establishes that hydrolysis of VLDL by LPL can activate PPARs. In macrophages, LPL-mediated activation of PPAR δ was reported while LPL action preferentially activated PPAR α in EC [10,82]. In cell-free systems, VLDL hydrolysis by LPL preferentially displaced high potency synthetic PPAR ligands from expressed PPAR proteins in the order of PPAR α >> PPAR δ >> PPAR γ . In different cellular settings, any of these effects may be relevant. Interestingly, not all fatty acid-releasing lipases had similar effects, as evident by secretory phospholipase A2 and LPL releasing the same amount of total fatty acids but having completely divergent effects on PPAR α [81]. This data underscores the complexity present among lipases, lipoproteins, and their lipolytic products and how such diversity may influence PPAR responses. In terms of atherosclerosis, this LPL/VLDL pathway repressed cytokine-induced VCAM-1 expression, reproducing the effects of synthetic PPAR α agonists. LPL-treated VLDL also induced expression of LPL, a known PPAR α target gene. The latter finding suggests possible feedback loops that may exist maintaining efficient handling of triglycerides. Certainly PPAR α

activation by the action of LPL would offer a mechanism through which enzymes of fatty acid beta oxidation and transport might be coordinately regulated. In mouse macrophages, incubation with fatty acids increased LPL mass and activity, as well as LPL mRNA levels. The parallel increase in binding of nuclear proteins to the PPAR responsive element in the LPL promoter was inhibited by immunoprecipitation with PPAR α antibody, suggesting that fatty acid-induced LPL expression requires PPAR α [83]. Of note, basal expression of PPAR α in murine macrophages appears marginal, a factor that must be kept in mind when considered experimental effects with PPAR α agonists in mouse models, with effects that could be PPAR α -independent [84]. PPAR α is expressed in human macrophages, where its activation increased LPL mRNA, mass, and activity [85]. In contrast, others have reported that in human macrophages, PPAR α activation may decrease secreted LPL mass and activity [86]. LPL is under complex regulation as is also evident by the existence of other proteins like ApoCII and apoAV (Figure 5), which both increase LPL activity [87,88], and apoCI and apoCIII, as well as the recently described members of the angiopoietin-like proteins Angptl3 and Angptl4, all of which can inhibit LPL activity [89–92]. It will be of interest to see how the multiple functional LPL polymorphisms known to exist in humans might result in clinical phenotypes as a result of changes in PPAR α activation. [93].

More recently, we reported that HDL hydrolysis by endothelial lipase could also activate PPAR α (Figure 5). Several aspects of this data suggest a pathway that is distinct from LPL/VLDL-mediated PPAR α activation [94]. HDL catabolism as a means of PPAR α activation offers an intriguing platform that may help explain the anti-inflammatory effects of HDL. Indeed, we found that the well-established ability of HDL to limit leukocyte adhesion to an activated endothelium was blocked in either the presence of a general lipase inhibitor as well as absent in EC from PPAR α -deficient mice. EL has been previously implicated as promoting atherosclerosis [95,96]. It is possible that EL-mediated PPAR α activation may have untoward effects, contributing to EL's pro-atherogenic effects. Alternatively, these studies may offer a subtle but potentially important distinction between the physiologic versus and pathologic effects of any given protein under study. Presumably, HDL hydrolysis by endothelial lipase exists in order to play some functional role in the appropriate setting(s). Recent failures with drugs that simply block enzymes of lipoprotein metabolism may offer a cautionary note regarding the loss of whatever functional role these mediators of lipid/lipoprotein metabolism play.

PPAR α Activation: Good or Bad?

Although the bulk of PPAR α data argues for PPAR α as a mechanism for limiting inflammation and atherosclerosis, it is important to note countering evidence that suggests PPAR α may also exert untoward effects. Although mouse bone marrow transplantation experiments of PPAR α deficiency in macrophages support PPAR α as decreasing atherosclerosis, crossing PPAR α deficient mice with ApoE-deficient mice led to more atherosclerosis rather than decreasing atherosclerosis as might have been predicted in PPAR α were protecting against atherosclerosis [97]. It remains unclear how to reconcile these results. More broadly, species differences may contribute to some of the discrepancies seen in PPAR α effects. For example, PPAR α agonists potentially decreased atherosclerosis in mice in which human apoAI was transgenically overexpressed [98]. PPAR α activation has also been implicated in cardiomyopathy, such as may occur through fatty acid toxicity in diabetes as well as in hypertension [99].

Given the data regarding PPAR α effects on pathways of lipid metabolism, inflammation and atherosclerosis in vitro and in vivo including surrogate markers in humans, clinical cardiovascular trials with fibrate drugs have been undertaken and closely considered for evidence that PPAR α activation might contribute to the responses seen. The Veterans

Administration-HDL Intervention Trial (VA-HIT) examined the effects of the PPAR α activating gemfibrozil versus placebo on cardiovascular events in a cohort of ~2500 patients with a prior history of cardiovascular disease, relatively average LDL levels, and low HDL/modestly elevated triglycerides. The study was positive, showing a significant decrease in cardiovascular events; subjects with diabetes appeared to particularly benefit from the fibrate treatment. Importantly, none of the subjects in VA-HIT were on a statin [100]. Given these observations, the results of the even larger FIELD trial, with ~10,000 subjects with diabetes treated with fenofibrate or placebo, were eagerly anticipated, especially given fenofibrate's greater potency as a PPAR α agonist than gemfibrozil. FIELD failed to show a difference in the primary cardiovascular endpoint. This lack of effect may have been due to the drop-in rate of statin use, which occurred disproportionately more among the placebo group and may have mitigated any difference in risk reduction [101]. A decrease in non-fatal MI and small vessel disease were seen as positive secondary endpoints. It is also possible the relatively higher level of HDL found among FIELD subjects may have also defined this group of subjects as being less likely to respond to a fibrate. Regardless, resolution of the FIELD data as it stands in comparison to VA-HIT is challenging. Statins remain first line drugs for reducing cardiovascular risk in patients with dyslipidemia and/or elevated LDL levels, and it may be challenging to add to this reduction in the clinical trial of typical duration and size. It is also possible that fibrates may not be the best way in which to target PPAR α for therapeutic benefit.

Conclusion

Atherosclerosis is a chronic disease characterized by lipid and fibrous depositions in the arterial wall in the setting of a chronic pro-coagulant, pro-inflammatory state. The potential importance of PPAR α in atherosclerosis is evident by its transcriptional regulation of pathways involved in atherogenic dyslipidemia, extracellular matrix remodeling, cholesterol efflux, thrombogenicity, and inflammation. As such, PPAR α may well be involved in all the stages of atherosclerosis, from the earliest fatty streaks to the late stage complicated lesions that can undergo plaque rupture and provoke myocardial infarction. Perhaps even more importantly, the involvement in PPAR α in so many aspects of atherosclerosis suggests this nuclear receptor may be involved in helping coordinate responses that connect energy balance, inflammation and atherosclerosis. Together the existing data establishes the importance of PPAR α in these critical pathways even while the impact of current PPAR α -activating drugs on improving cardiovascular outcomes remains under debate. In general, most of the available evidence suggests PPAR α activation regulates various targets that should decrease atherosclerosis and its complications. More research will be required in order to fully understand how PPAR α regulates and more importantly integrates control of multiple mechanisms involved in atherosclerosis, and how best to target PPAR α for therapeutic benefit.

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Figure 1. PPAR α activation modulates the hepatic acute phase response

Hepatic expression of inflammatory cytokines – such as CRP, fibrinogen and SAA – is inhibited by PPAR α , both under basal conditions and after stimulation with cytokines such as IL-1, IL-6 and TNF α from the circulation. CRP, C-reactive protein; IL-1, interleukin-1; IL-6, interleukin-6; SAA, serum amyloid A; TNF α , tumor necrosis factor α .

Figure 2. PPARα activation modulates reactivity of the vascular endothelium

Activation of PPARα in ECs decreases production and secretion of ET-1, which would be expected to decrease vasoconstriction and SMC proliferation. PPARα has been reported to increase levels of endothelial nitric oxide synthetase (eNOS), which is the enzyme responsible for NO generation. ENOS induction would be expected to increase NO production and thus increase vasodilatation. Increased NO decreases expression of VCAM-1, a key player in leukocyte adhesion to the endothelium. PPARα activation can directly decrease VCAM-1 expression, which is thought to involve inhibition of NFκB activity. eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; NO, nitric oxide; SMC, smooth muscle cell; VCAM-1, vascular cell adhesion molecule-1.

Figure 3. PPAR α inhibits local inflammatory responses in the endothelium

PPAR α activation in the vasculature inhibits adhesion of T-cells and dendritic cells to the endothelium through inhibition of VCAM-1 expression. PPAR α activation also decreases expression of inflammatory cytokines such as IFN- γ , TNF α , IL-2 by Th1 cells. In addition, PPAR α inhibits the induction of COX2 expression by SMCs activated in response to IL-1 β . COX2, cyclooxygenase 2; IFN- γ , interferon- γ ; IL-2, interleukin 2.

Figure 4. PPARα activation modulates atherosclerotic plaque formation at multiple levels

In addition to its effects on VCAM-1 expression, PPARα activation also purportedly limits macrophage foam cell formation by inhibiting oxidative modification of LDL and by promoting reverse cholesterol transport via increased SR-BI and ABCA1 expression. PPARα activation may also decrease plaque thrombogenicity by inhibiting expression of the procoagulant tissue factor (TF). PPARα activation may also repress expression of MMP9, an enzyme implicated in plaque rupture, and decrease SMC proliferation. MMP9, matrix metalloproteinase.

Figure 5. Specific pathways of lipoprotein metabolism activates PPARα

VLDL and HDL hydrolysis by LPL and EL respectively can generate PPARα activation. In response to the action of these enzymes on these lipoprotein substrates, PPARα target genes are regulated, including targets implicated in atherosclerosis, like VCAM-1. When these lipolytic pathways are integrated into PPAR responses, the possibility that other lipase regulators, like ApoCII and ApoAV, as well as LPL inhibitors like ApoCI, ApoCIII, Angptl3 and Angptl4 may also regulate PPAR activity becomes apparent. Angptl, angiopoietin-like protein; Apo, apoprotein; EL, endothelial lipase; HDL, high density lipoprotein; LPL, lipoprotein lipase; VLDL, very low density lipoprotein.