PPAR-α effects on the heart and other vascular tissues

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Francis, Gordon A., Jean-Sébastien Annicotte, and Johan Auwerx. PPAR-α effects on the heart and other vascular tissues. Am J Physiol Heart Circ Physiol 285: H1–H9, 2003. First published March 6, 2003; 10.1152/ajpheart.01118.2002.—Peroxisome proliferator-activated receptor (PPAR)-α is a member of a large nuclear receptor superfamily whose main role is to activate genes involved in fatty acid oxidation in the liver, heart, kidney, and skeletal muscle. While currently used mainly as hypolipidemic agents, the cardiac effects and anti-inflammatory actions of PPAR-α agonists in arterial wall cells suggest other potential cardioprotective and antiatherosclerotic effects of these agents. This review summarizes current knowledge regarding the effects of PPAR-α agonists on lipid and lipoprotein metabolism, the heart, and the vessel wall and introduces some of the insights gained in these areas from studying PPAR-α-deficient mice. The introduction of new and more potent PPAR-α agonists will provide important insights into the overall benefits of activating PPAR-α clinically for the treatment of dyslipidemia and prevention of vascular disease.

atherosclerosis; fatty acid oxidation; lipoproteins; cholesterol; nuclear receptors; peroxisome proliferator-activated receptor-α; ATP-binding cassette transporter A1; fibrate; high-density lipoprotein

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metabolic processes. PPAR-α activity is also enhanced by protein kinase A-dependent phosphorylation (51).

CLINICAL USE OF PPAR-α AGONISTS

PPAR-α agonists in the form of fibric acid derivatives or fibrates (clofibrate, gemfibrozil, fenofibrate, bezafibrate, and cipofibrate) have been in use for over 40 yr for the treatment of dyslipidemia, mainly due to their actions of lowering triglyceride (TG) levels, raising high-density lipoprotein (HDL), and the more recently recognized effect of decreasing levels of small, dense low-density lipoprotein (LDL) particles (24, 81). The Helsinki Heart Study was the first large clinical trial to show benefits from the use of a fibrate, demonstrating a 34% reduction in the overall cardiac event rate in men with dyslipidemia treated for 5 yr with gemfibrozil (30). The more recent Veterans Affairs HDL Cholesterol Intervention Trial demonstrated a 22% reduction in coronary events and mortality in men with low HDL as their primary lipid abnormality and treated for over 5 yr with gemfibrozil (72). Although attributed mainly to the hypolipidemic and HDL-raising actions of fibrates, other actions of these PPAR-α agonists on blood vessels, thrombotic factors, and possibly the heart itself may also have contributed to the benefits seen in these trials.

The success of these trials has provided an increased recognition of the importance of nuclear receptors, including PPAR-α, as master regulators of genes involved in metabolic control at several levels. This awareness has resulted in an intensive search for novel activators of these receptors that might be used in preventive and therapeutic strategies to combat common diseases such as atherosclerosis, diabetes, obesity, and possibly some forms of heart disease. The following sections review the current understanding of the effects of PPAR-α agonists in various tissues and the potential benefits and drawbacks associated with broader use of these agents to treat and prevent cardiovascular disease. The major actions of PPAR-α on lipid homeostasis and the cardiovascular system are summarized in Fig. 1.

PPAR-α EFFECTS ON TG, LDL, AND HDL METABOLISM

The most clearly defined actions of PPAR-α agonists are those at the hepatic and extrahepatic levels on lipoprotein metabolism. PPAR-α is the primary PPAR subtype expressed in the liver and is a major regulator of the hepatic metabolism of fats, synthesis and catabolism of lipoproteins, and some steps in the HDL synthetic and reverse cholesterol transport pathways. Binding to PPAR-α by fatty acid, eicosanoid, and fibrate drug ligands leads to activation of numerous genes involved in β-oxidative catabolism of fatty acids (26, 42, 44). In addition to inducing all the genes encoding enzymes of the classical fatty acid β-oxidation pathway [e.g., acyl-CoA oxidase, very-long-chain and medium-chain acyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase (66)], PPAR-α also activates the genes necessary for cellular uptake of fatty acids (fatty acid transport protein) and their initial derivatization for entry into the β-oxidation cycle (acyl-CoA synthetase) (for a review, see Ref. 76). PPAR-α also stimulates FAO by these enzymes in the other tissues in which it is expressed, including the heart, skeletal muscle, and kidney (5, 78). Increased diversion of fatty acids into β-oxidation decreases the availability of fattyacyl CoA substrates for TG synthesis and therefore decreases very-low-density lipoprotein (VLDL) secretion by the liver. The relative importance of hepatic peroxisomal FAO induced by PPAR-α agonists in humans, which have an order of magnitude less PPAR-α mRNA in the liver compared with rodents, is not yet clear. Hepatic mitochondrial, rather than peroxisomal, β-oxidation of fatty acids is likely the major site of PPAR-α action in the human liver, as seen in the heart (94). The lower expression of PPAR-α in the liver and the lack of a peroxisome proliferative response in humans may be the major reason the fibrates have not been associated with hepatocarcinogenesis in humans as they have in rodents (36).

In addition to limiting substrate availability for TG and VLDL synthesis, PPAR-α has been reported to inhibit expression of apolipoprotein (apo) C-III (34, 84), a protein that inhibits both the TG-hydrolyzing action of lipoprotein lipase (LPL) and the uptake of TG-rich lipoprotein remnants (80, 99). Inhibition of apo C-III expression would therefore enhance TG-rich particle hydrolysis and improve the uptake of their remnants by the liver, thereby decreasing plasma TG levels further. Although active in inhibiting apo C-III in rodents, the ability of PPAR-α agonists to lower apo C-III in humans remains controversial (38, 50). PPAR-α agonists may also decrease TG levels by increasing the expression of LPL in the liver (77) and in macrophages (2, 60). How much of the hypotriglyceremic effect of PPAR-α ligands can be ascribed to direct versus indirect effects (via decreased apo C-III) on LPL is not yet known.

The fibrate drug class of PPAR-α agonists also decreases TG content of LDL particles, converting them from the more atherogenic small, dense phenotype to larger, less atherogenic LDL (55, 101). This effect is likely due to the combined effects of lower VLDL and TG and increased VLDL remnant clearance and therefore lower TG levels available for transfer to LDL.

In addition to beneficial effects on fatty acid and TG metabolism, PPAR-α also enhances components of the HDL synthetic pathway. Synthesis of apo A-I and apo A-II, the two major proteins of HDL, by the liver and intestine is the first step in HDL particle formation. PPAR-α activation by fibrates activates human apo A-I and apo A-II genes in the liver, leading to increased synthesis of these proteins (95–98). A recent study (36) using rabbits expressing the human apo A-I transgene along with its PPRE showed an increase in human apo A-I mRNA and mass in response to fenofibrate treatment in the absence of any peroxisome-proliferative effect of the fibrate.

The second mechanism for increasing HDL-cholesterol concentrations in plasma is the delivery of redun-
dant surface phospholipid and apolipoprotein components of VLDL and chylomicrons onto HDL during hydrolysis of TG-rich particles by LPL (35). Enhancement of TG hydrolysis, by PPAR-α-induced reduction in apo C-III synthesis and an increase in LPL synthesis and activity, decreases TG levels, increasing the transfer of other surface components of TG-rich particles to HDL (6, 35). HDL synthesis is also enhanced by the actions of the phospholipid transport protein (PLTP), which may explain a portion of the HDL-raising effect of fibrates because they increase expression of PLTP by PPAR-α (8).

The third and rate-limiting mechanism of HDL formation is the removal of cellular phospholipids and cholesterol by lipid-free or lipid-poor HDL apolipoproteins (63). The importance of this pathway was highlighted by the discovery that, despite normal synthesis of HDL apolipoproteins (43, 57), cultured cells isolated from patients with the low HDL syndrome Tangier disease failed to release both phospholipids and cholesterol to apo A-I (28, 68). The gene defect responsible for Tangier disease was identified in 1999 by several groups in the ATP-binding cassette transporter AI (ABCA1) (for a review, see Ref. 62). Regulation of this membrane transporter, currently thought to mediate the delivery of either phospholipid alone or both phospholipid and cholesterol to apo A-I, is in a large part by nuclear receptors including the liver X receptor (LXR) and the PPARs (18, 69, 79, 93). Some studies suggest both PPAR-α and PPAR-γ activate ABCA1 expression indirectly by enhancing the transcription of LXR-α, the promoter of which contains a PPRE (13, 16, 49, 90). A recent study, however, found no change in liver ABCA1 mRNA levels in PPAR-α knockout mice (48). While additional studies are required, these findings lend further support for the use of synthetic PPAR as well as LXR or RXR agonists in the prevention or treatment of atherosclerosis.

The final uptake of cholesterol carried on HDL by the liver is mediated through the concerted actions of scav-
PPAR-α AND THE HEART

In contrast to the clearly beneficial effects of PPAR-α agonists on lipoprotein metabolism, the net effects of PPAR-α activation on cardiac metabolism and function in normal and disease states are less well defined. The main activity of PPAR-α in the heart is to provide energy to the myocardium by activating genes regulating mitochondrial fatty acid uptake and oxidation (94). The fetal heart relies primarily on glucose and lactate as energy sources. After birth, the capacity for PPAR-α-dependent FAO increases markedly. This results in the heart using FAO as the preferred substrate for ATP production while retaining the ability to switch to glucose and lactate utilization to meet its energy demands with varying dietary and physiological conditions (74, 94). Studies using cultured neonatal cardiomyocytes have demonstrated the direct effects of fatty acids and PPAR-α agonists in activating PPAR-α-dependent enzymes of fatty acid uptake and FAO in the heart (10, 91, 92). In vivo studies in adult rats showed that expression of myocardial uncoupling protein 3, a PPAR-α-dependent enzyme, was increased on a high-fat diet and decreased on a low-fat diet (103). These results suggest that the circulating levels of TGs and fatty acids are important determinants of substrate availability for myocardial PPAR-α and FAO.

A major controversy regarding changes in PPAR-α activity in cardiac disease states is whether these are adaptive or causally related to myocardial pathology. In conditions of pressure-induced cardiac hypertrophy, PPAR-α is downregulated, resulting in reversion of the heart to the fetal pattern of glucose and lactate substrate utilization (4, 74). Diminished cardiac FAO and increased utilization of glucose has been found in studies of both murine (73) and human (19) cardiac hypertrophy. This switch reduces the oxygen requirement of the heart to produce ATP, which is higher per mole of fatty acid substrate oxidized than for glucose (94). In this sense, the shift from PPAR-α-dependent FAO to glucose utilization can be considered an adaptive response (29). In the long term, however, this switch becomes detrimental as less ATP is generated per mole of glucose oxidized, and lipid accumulation and lipotoxicity of the myocardium may develop (5). Whether this switch of energy substrates by deactivation of PPAR-α is actually an adaptive response to pressure overload or a mediator of the development of heart failure in pressure-induced cardiac hypertrophy, due to decreased ATP production and lipid accumulation, is not yet known (53). In a murine model of pressure-overload cardiac hypertrophy, reactivation of PPAR-α by agonists prevented substrate switching to glucose and resulted in severe depression of cardiac power and efficiency in the hypertrophied heart (102). The conclusion from this study was that PPAR-α downregulation is essential for the maintenance of contractile function of the hypertrophied heart. The significance of these findings to human cardiac hypertrophy is not yet known. To date, there are no reports of worsening of cardiac function in human cardiac hypertrophy with the use of PPAR-α agonist fibrates. The lack of such reports suggests any detrimental direct effects of PPAR-α agonists on cardiac function in cardiac hypertrophy may be offset by the beneficial effects of decreasing TG and free fatty acid substrate availability to the ailing myocardium, thereby decreasing lipid accumulation in hearts undergoing a switch to increased glucose utilization.

In contrast to the alterations in substrate use by the hypertrophied myocardium, the heart in uncontrolled diabetes is constrained from switching to glucose oxidation due to lack of insulin or insulin resistance, resulting in impaired glucose utilization and an almost exclusive use of FAO to provide the ATP needs of the myocardium (71, 85). Increased myocardial fatty acid uptake and reliance on FAO for energy production results in increased myocardial oxygen consumption due to excessively high mitochondrial oxidative flux. High circulating levels and uptake of TG and free fatty acids by the diabetic heart leads to excess myocardial lipid accumulation, which further predisposes to contractile dysfunction and myocyte death (17, 104). Whether this is the fault of PPAR-α induction of FAO, or simply a reflection of the altered substrate availability in the diabetic heart, is unknown. If, however, excess fatty acid substrates are the culprit, a reduction of circulating TG and free fatty acid levels induced by PPAR-α agonists such as fibrates would likely have a net beneficial effect on the diabetic myocardium. Aasum et al. (1) found that treatment of diabetic db/db mice with a PPAR-α agonist normalized circulating free fatty acid, TG, and glucose levels, actually reduced myocardial FAO by 50%, and increased myocardial glucose utilization. They also found that FAO enzymes were not upregulated by the PPAR-α agonist in these mouse hearts. These results suggested that the extra-cardiac effects of the PPAR-α agonist, by decreasing circulating free fatty acid levels and increasing insulin sensitivity, have greater effects on cardiac energy utilization by decreasing the activation of PPAR-α and the substrate availability of FAO enzymes rather than directly inducing their expression in the heart. This conclusion is consistent with the requirement of fatty acid substrates to activate PPAR-α-dependent fatty acid transporters MDR2 could be a final point where PPAR-α agonists affect reverse cholesterol transport, by favoring the excretion of phospholipids and associated cholesterol from the liver into bile (48).
acid uptake and FAO by cultured neonatal cardiomyocytes (10, 91, 92) and the direct correlation between UCP-3 expression in adult rat hearts and dietary fat intake by these animals (103). Although the Aasum et al. study (1) also showed a 46% decrease in myocardial TG content after a 4- to 5-wk treatment with a PPAR-α agonist, they did not show improvements in contractile function of the myocardium. In contrast to this study, which started agonist treatment at 8 wk of age, Zhou et al. (104) found decreased myocardial TG content and improved myocardial function in Zucker diabetic fatty rats treated with a PPAR-γ agonist for 6 or 13 wk beginning at 6–7 wk of age. The ability of PPAR-α agonists to prevent or reverse structural changes in the diabetic myocardium may therefore depend on earlier or more prolonged treatment with the agonist. Further studies are required to answer this question.

In contrast to these findings suggesting that substrate availability is the key determinant of the activity of PPAR-activated FAO genes in the myocardium, Finck et al. (25) showed that cardiac-specific overexpression of PPAR-α increased FAO, decreased glucose utilization, and induced a diabetic-type cardiomyopathy in otherwise normal mice. The fact that these changes occurred even on a normal chow diet in non-diabetic animals may argue against substrate availability being a key determinant of PPAR-α function in the heart. The constitutive overexpression of PPAR-α in the hearts of these animals, however, and their inability to utilize glucose as an energy source means they cannot be compared with diabetic mice capable of increasing glucose utilization in response to improved insulin sensitivity, as seen with the use of a PPAR-α agonist in the Aasum et al. study (1). Final conclusions about the overall importance of direct versus indirect (noncardiac) effects of PPAR-α agonists on the heart await further studies.

The net effects of PPAR-α agonists on the ischemic heart are also still unclear. Whereas some studies demonstrated the beneficial effects of pretreatment with PPAR-α agonists on the degree of ischemic injury, others emphasized the importance of the energy switch away from PPAR-α-induced FAO in the postischemic heart to decrease the oxygen cost of energy production from fatty acids. Tabernerio et al. (87) showed that pretreatment of control mice with fenofibrate for 10 days reduced infarct size and improved postischemic contractile dysfunction using an ischemia-reperfusion injury model, whereas PPAR-α-null mice were more susceptible to the ischemic injury and refractory to protection by fenofibrate. Wayman et al. (100) also found that pretreatment with both PPAR-α and PPAR-γ agonists for just 30 min before ischemic injury resulted in a substantially decreased postischemic myocardial infarct size in rats. Explanations for these findings include improvements in the metabolic milieu (decreased free fatty acid and TG levels) before ischemia, improved ATP stores before ischemia, and decreased expression of the proinflammatory markers nuclear factor (NF)-κB and activator protein (AP)-1 (see PPAR-α EFFECTS AND THE VESSEL WALL) after ischemia.

Agents that diminish FAO and increase glucose utilization after the ischemic insult have been found to improve myocardial recovery postischemia (56). The rationale in this case is that glucose utilization requires less oxygen consumption for energy generation than does FAO and does not worsen acidosis in the damaged myocardium the way FAO can. Severe ischemia itself turns off PPAR-α-regulated gene expression as a source of ATP production from fatty acids (39, 65). Glucose transporter GLUT4-deficient mice develop profound myocardial dysfunction during ischemia (89), suggesting the ability to switch to glucose utilization after ischemia is critical (56). Overall, it appears that treatment with PPAR-α agonists preischemia may limit ischemic damage to the myocardium but that postischemia the physiological or treatment-induced (using agents such as trimetazidine or dichloroacetate (56)) switch from FAO to glucose utilization improves cardiac efficiency and aids in recovery of the damaged myocardium.

The heart also expresses LPL, which has been found to be upregulated in the liver and macrophages in response to PPAR-α agonists (60, 77). Although likely a key player in myocardial TG hydrolysis and fatty acid uptake, cardiac LPL activity has been found to be inhibited by PPAR-α agonists in cultured rat cardiomyocytes (12). These results suggest that PPAR-α activation by fatty acids or agonists could actually inhibit LPL as a protective mechanism against a potentially toxic oversupply of fatty acids to the myocardium (12).

In summary, it appears that PPAR-α is vital as an activator of FAO and energy production by the heart but that PPAR-α agonists might decrease net FAO in the heart due to decreasing circulating TG and free fatty acid levels, i.e., by limiting substrate availability for PPAR-α-activated FAO enzymes (Fig. 1). Pretreatment with PPAR-α agonists may improve the outcome of cardiac ischemic events; however, it seems likely these agents would be contraindicated in the immediate postischemic period if they reverse the switch to glucose utilization during recovery of the damaged myocardium. Further studies are required to more clearly define the role of PPAR-α in the pathogenesis of cardiac hypertrophy, myocardial substrate utilization, and protecting against postischemic injury.

**PPAR-α AND THE VESSEL WALL**

PPAR-α agonists have been found to have numerous potentially beneficial actions in cultured artery wall cells, suggesting further antiatherosclerotic activities of these agents in addition to their beneficial effects on lipids and lipoproteins (7, 94). Nonlipid-related effects on atherosclerosis were also suggested by studies in cholesterol-fed rabbits where fibers decreased atherosclerosis without appreciable effects on circulating lipid levels (for a review, see Ref. 94). PPAR-α is expressed in human aortic smooth muscle cells and mediates inhibition of interleukin (IL)-6 and prostaglandin production and expression of cyclooxygenase 2 (82). The latter effect is due to PPAR-α repression of NF-κB.
signaling. This effect also inhibits IL-6 secretion by activated endothelial cells (20), which also express PPAR-α, thereby decreasing IL-6-dependent induction of monocyte chemotactic protein 1 expression. Clinically, fibrate treatment reduces circulating IL-6 levels as well as the prothrombotic factor fibrinogen and the marker of inflammation C-reactive protein (82). Tumor necrosis factor-α-induced expression of vascular cell adhesion molecule 1 by endothelial cells, an effect also mediated through NF-κB signaling, is also inhibited by PPAR-α agonists (59). Tissue factor expression by monocytes, a major component of thrombus formation in acute coronary events, is also inhibited by PPAR-α agonists (61). This effect is thought to be mediated by inhibition of NF-κB and AP-1 (59, 61). PPAR-α-dependent inhibition of AP-1 is likewise thought to mediate the inhibition of expression of endothelin 1, a potent inducer of cell adhesion molecules expression after endothelial injury (21). Macrophage production of scavenger receptor A, a mediator of uptake of oxidized LDL and therefore of foam cell formation, and of matrix metalloproteinase 9, a mediator of cell invasion from the vessel wall into the intima, are also inhibited by PPAR-α agonists (94). In addition, fibrates have been shown to reduce plasma levels of fibrinogen, which in vivo would reduce the likelihood of thrombogenesis (47). Clearly, PPAR-α agonists are likely to have numerous beneficial antiatherosclerotic actions in addition to their defined benefits on lipid and lipoprotein metabolism.

LESSONS FROM PPAR-α KNOCKOUT MICE

The generation of PPAR-α-null mice has helped to confirm several of the proposed actions of this nuclear receptor as well as identifying new actions. These mice are viable, fertile, and exhibit no gross phenotypic defects (52). They do, however, exhibit profound metabolic abnormalities in the liver and heart. These mice fail to exhibit peroxisome proliferation or activation of FAO target genes when exposed to PPAR-α agonists (52, 64, 87). They accumulate increased hepatic TG in response to feeding and during fasting (64, 86). PPAR-α-null mice develop severe hypoglycemia when fasted (45), due to impaired FAO and increased reliance on glucose as an energy source. This also likely explains their resistance to high-fat diet-induced insulin resistance (33). These mice also develop massive accumulation of myocardial lipids under conditions that increase fatty acid flux, confirming the importance of PPAR-α in mediating FAO and preventing the toxic accumulation of fat in the heart (22). Campbell et al. (11) showed that the marked increase in malonyl-CoA, a potent inhibitor of FAO, in the hearts of PPAR-α-null mice is due to decreased expression of malonyl-CoA carboxylase. These results demonstrate yet another mechanism of regulation of FAO by PPAR-α.

In conclusion, it is now well established that PPAR-α agonists have beneficial effects clinically in lipid metabolism and in decreasing coronary vascular events and mortality. In vitro and animal studies now also suggest numerous direct anti-inflammatory and antiatherosclerotic effects of PPAR-α agonists in the artery wall. Less certain are the overall effects of PPAR-α on the development of pressure-induced and diabetic cardiomyopathy and the role of PPAR-α agonists in preventing postischemic myocardial injury. Drugs under development with enhanced PPAR-α agonist or combined PPAR-α/PPAR-γ activity will further help in identifying the benefits of activating this receptor clinically, but will need to be assessed critically for potentially detrimental effects in situations such as cardiac ischemia.

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REFERENCES


