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ARTICLES

Modulation of PPARγ Activity With Pharmaceutical Agents: Treatment of Insulin Resistance and Atherosclerosis

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Abstract The anti-diabetic thiazolidinediones (TZDs) are a class of compounds with insulin-sensitizing activity that were originally discovered using in vivo pharmacological screens. In subsequent binding studies, TZDs were demonstrated to enhance insulin action by activating peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a member of the ligand-activated nuclear receptor superfamily that promotes adipogenesis and enhances insulin sensitivity by controlling the expression of genes in glucose and lipid metabolism. Given the large size of the ligand binding pocket in PPARγ, novel classes of both full and partial agonists that are structurally distinct from TZDs have been discovered. These compounds have been effective tools in differentiating adipogenic and insulin-sensitizing activities as well as tissue selectivity of PPARγ activation. This information has led to the hypothesis that one ligand can activate or inactivate PPARs depending upon the tissue in which the PPAR resides. Thus particular compounds can be designated selective PPAR modulators or SPPARMs, a concept similar to that observed with the activation of estrogen receptor (ER) by SERMS. Additionally, both preclinical and clinical data suggest that PPARγ activation is useful for the prevention of atherosclerosis. However, the effects of TZDs on plasma lipid profiles do not solely account for their anti-atherogenic effects. Recent studies with macrophage cells and animal models for atherosclerosis indicate that TZDs reduce the size and number of lesions formed in the vessel wall by modulating foam cell formation and inflammatory responses by macrophages. Thus in addition to the treatment of type II diabetes, PPARγ agonists can be potentially employed for the treatment of atherosclerosis in general population. J. Cell. Biochem. 89: 38–47, 2003.

Key words: PPARγ; insulin resistance; atherosclerosis; TZD; agonist; NIDDM; PPRE

Type II diabetes or non-insulin-dependent diabetes mellitus (NIDDM) is characterized by insulin resistance and impaired glucose tolerance. In the early stages of the disease, increased hepatic glucose output and decreased glucose disposal in the peripheral tissues cause elevation of plasma glucose levels. To compensate for the elevated glucose levels and insulin resistance, pancreatic β-cells secrete higher levels of insulin causing hyperinsulinemia. Continued escalation of insulin secretion and the insulin resistance eventually lead to failure of the β-cell and frank type II diabetes. Insulin resistance and hyperinsulinemia are often associated with dyslipidemia, hypertension, atherosclerosis, and obesity, a collection of metabolic abnormalities characterized as Syndrome X [Reaven, 1988, 1991; DeFronzo and Ferrannini, 1991]. Coronary heart disease (CHD), as a result of atherosclerosis, is a major cause of death in NIDDM patients [Garcia et al., 1974]. Although, the exact cause of increased atherosclerosis is not clear, improvement of the metabolic disorders characterized by Syndrome X can significantly reduce risks. Improvement of insulin action and reduction of plasma lipid and blood glucose levels have been the primary means to reduce risks of CHD. In addition, direct intervention of plaque formation on the artery wall would provide major clinical value in preventing atherosclerosis. A therapeutic agent

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offering both insulin-sensitizing and antiatherogenic activities is ideal for the treatment of type II diabetic patients.

PPARγ AGONISTS IN THE TREATMENT OF TYPE II DIABETES: MECHANISM OF ACTION

Thiazolidinediones (TZDs) are a class of antidiabetic compounds that improve insulin sensitivity in various animal models of diabetes and obesity [Saltiel and Olefsky, 1996; Olefsky and Saltiel, 2000]. The original lead for TZDs was clofibrate, a compound with antilipidemic and weak antihyperglycemic activity in human [Barnett et al., 1977]. Based on the structure of clofibrate, in vivo screening for increased antihyperglycemic activity generated ciglitazone, the first TZD [Kawamatsu et al., 1980; Sohda et al., 1982]. Continued analog synthesis and in vivo screening led to the identification of more TZDs with increased potency [Hulin, 1994], including troglitazone, rosiglitazone (BRL49653), englitazone, and pioglitazone. In studies with obese-diabetic yellow KK mice, ciglitazone markedly reduced insulin resistance, improved insulin sensitivity, and suppressed diabetic syndromes in these animals [Fujita et al., 1983]. It also improved glucose tolerance in obese Zucker fatty rats in similar studies [Fujita et al., 1983]. Although ciglitazone had no effect on glucose and lipid metabolism of young Sprague–Dawley rats, it was effective in old Sprague–Dawley rats that had moderate insulin resistance and hyperlipidemia [Fujita et al., 1983]. These data suggest that ciglitazone is only effective on abnormal glucose and lipid metabolism associated with insulin resistance. This finding is consistent with a similar observation that englitazone did not produce overt hypoglycemia in non-diabetic animals but reduced plasma glucose and insulin levels in diabetic animal models [Fujiwara et al., 1988; Stevenson et al., 1990, 1991]. Troglitazone, with half of its structure from vitamin E, serves both as an antidiabetic agent and an antioxidant [Nolan et al., 1994; Noguchi et al., 1996]. In clinical trials, troglitazone improved glucose tolerance in obese subjects and reduced insulin resistance in non-obese diabetic patients [Johnson et al., 1998]. Although TZDs also reverse insulin resistance induced by environmental factors such as diet, their effects are limited. For example, troglitazone is effective on insulin resistance induced by fructose but not by fat [Lee et al., 1994; Khoursheed et al., 1995].

The treatment of insulin resistance with TZDs generated tremendous interest in understanding the molecular mechanisms that underlie the pharmacological actions of this class of compounds. Several research groups identified peroxisome proliferator-activated receptor gamma (PPARγ), a member of the nuclear receptor superfamily of ligand-activated transcription factors as the receptor for TZDs [Ibrahimi et al., 1994; Tontonoz et al., 1994; Schoonjans et al., 1996]. There are three PPAR isoforms, PPARα, PPARβ or δ, and PPARγ. The amino acid sequence for the DNA binding domains for the different isoforms is highly conserved, with the most variation in the receptors being in the ligand binding domain. This sequence variation is responsible for the distinct ligand binding profile of each isoform. Upon ligand binding, activated PPARs form heterodimers with retinoic X receptor (RXR) and bind to peroxisome proliferator responsive elements (PPRE), which consist of a hexameric nucleotide direct repeat of recognition motif spaced by one nucleotide and has been identified in a large number of genes involved in lipid metabolism [Kliewer et al., 1995]. There are two isoforms of PPARγ, PPARγ1 and PPARγ2, which are generated by alternative splicing and promoter usage [Schoonjans et al., 1996]. PPARγ2 has extra 30 amino acids in the amino terminus. Although PPARγ1 is ubiquitously expressed, PPARγ2 is predominantly expressed in adipose tissue, where comparable level of PPARγ1 is expressed [Schoonjans et al., 1996]. The PPARγ and RXR complex, along with other co-activators for transcription, activate transcription of target genes [Kliewer et al., 1995] (Fig. 1). Naturally occurring fatty acids and eicosanoids, such as prostaglandin J derivatives, linoleic acid, and HODEs (9- and 13-), have been proposed as the natural ligands of PPARγ [Hallakou et al., 1997; Kliewer and Wilson, 1998]. Several research groups have shown that TZDs bind PPARγ in in vitro ligand binding assays and transactivate target genes under the control of PPREs in cell-based reporter assays [Ibrahimi et al., 1994; Tontonoz et al., 1994; Lehmann et al., 1995]. In addition, PPARγ agonists including TZDs activate adipocyte gene expression and promote adipocyte differentiation [Tontonoz et al., 1994; Lehmann

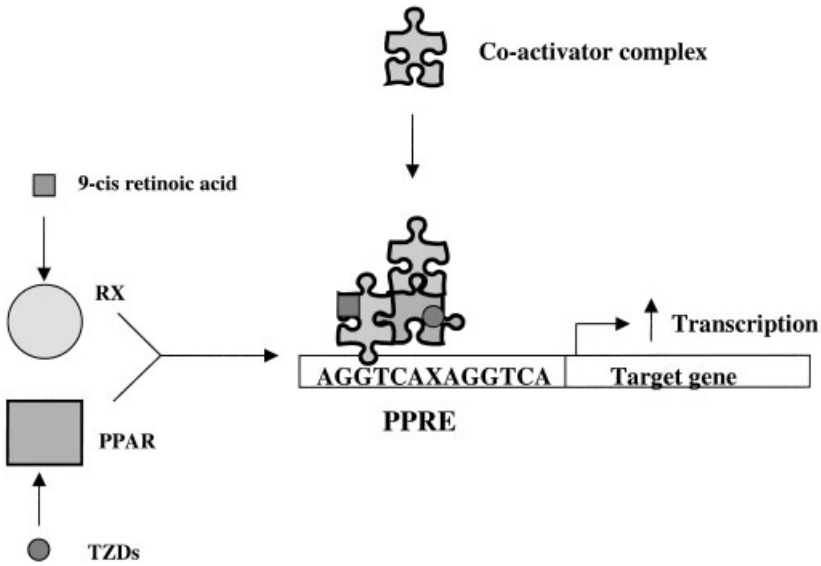

Fig. 1. Activation of proliferator-activated receptor gamma (PPARγ) by thiazolidinediones (TZDs). Upon binding by TZDs, PPARγ heterodimerizes with RXR, which is activated by 9-*cis* retinoic acid, becomes associated with co-activator complex, binds to PPREs and stimulates the expression of target genes.

et al., 1995]. Human patients with a dominant negative PPARγ mutation exhibit severe insulin resistance [Barroso et al., 1999].

The pharmacological mechanisms that underlie the antidiabetic effect by activation of PPARγ, a master regulator of adipocyte differentiation, are not directly related to the insulin signaling pathway. PPARγ agonists enhance both amino acid and glucose uptake in adipocytes [Tafuri, 1996; Su et al., 1998]. In white adipose tissue (WAT), TZDs induced marked decrease in the level of tumor necrosis factor α (TNFα), a signaling molecule secreted by adipocytes and associated with insulin resistance [Hallakou et al., 1997; Okuno et al., 1998]. The increased understanding of the adipose tissue for its role in regulating energy homeostasis has helped further explain the mechanisms of the insulin-sensitizing action of PPARγ [Ahima and Flier, 2000]. Two proteins synthesized in the adipose tissue, ACRP30 and resistin, are functionally linked to insulin sensitivity and regulated by TZDs. The circulating protein ACRP30, which is synthesized in adipose tissue and enhances insulin action upon injection in mice [Berg et al., 2001], is induced by PPARγ agonists [Combs et al., 2002]. While resistin, a fat cell-specific hormone that impairs insulin action and glucose tolerance in normal mice, is downregulated by TZDs [Steppan et al., 2001]. In addition, TZDs increased the number of small adipocytes and decreased the number of large adipocytes [Hallakou et al., 1997; Okuno et al., 1998]. Since small adipocytes are more metabolically active, more glucose is utilized as a result of TZD treatment. A most recent report demonstrated that TZDs stimulates glycerol kinase expression in adipocytes and consequently glycerol incorporation into triglyceride in fat cells, which may contribute to reduced plasma free fatty acid levels and improve insulin sensitization [Guan et al., 2002]. Thus, the adipose tissue seems to be important for glucose-lowering effects of TZDs. However, in tissues other than adipose, PPARγ activation by TZDs could also alleviate insulin resistance and reverse hyperglycemia [Burant et al., 1997]. Burant et al. [1997] assessed TZD action in mice that lack adipose tissue. These investigators found that troglitazone treatment ameliorated insulin resistance and lowered glucose levels, suggesting that TZDs can modulate glucose homeostasis via adipose-independent mechanisms. Based on this result, it has been proposed that TZDs may exert their functions in skeletal muscle, the major site for glucose disposal, although there is 10–100-fold lower PPARγ expression in this tissue [Park et al., 1997, 1998; Kruszynska et al., 1998; Camp et al., 2000]. However, it is important to note that increased PPARγ expression in skeletal muscle is associated with insulin resistance [Park et al., 1997; Kruszynska et al., 1998; Loviscach et al., 2000]. This discrepancy with the improved insulin

sensitivity by the activation of PPARγ by TZDs can be reconciled by the hypothesis that the normal role of PPARγ bound by endogenous ligands is to dampen insulin action through transcriptional repression [Miles et al., 2000; Olefsky and Saltiel, 2000]. TZDs reverse this repression and restore insulin sensitivity [Miles et al., 2000; Olefsky and Saltiel, 2000]. Taken together, the coordinated effects of activated PPARγ on target gene expression in multiple tissues, including fat and skeletal muscle, most likely explain the pharmacological action of TZDs. These data suggest that PPARγ activation increases insulin sensitivity both directly in target tissues and indirectly through adipose-specific regulatory proteins.

It is important to understand the individual roles of PPARγ1 and PPARγ2 in adipocyte differentiation. It is also critical to differentiate the mechanism of the surprisingly positive effect of reduced PPARγ activity on insulin sensitivity from that observed with PPARγ activation. For example, is there a distinct role for insulin sensitization or adipogenesis for each of the PPARγ isoforms in this tissue? Further, the ligand-dependent activation domain of PPARγ2 is 5–10-fold more effective than that of PPARγ1. Does the activation of the isoforms have different functional outcomes? The evidence from PPARγ knockout animals and human genetic studies complicates this simple question. Three independent research groups have made consistent findings that homozygous PPARγ knockout is lethal whereas heterozygous knockout mice are protected from the development of insulin resistance [Barak et al., 1999; Kubota et al., 1999; Miles et al., 2000]. However, the protection was not achieved by promotion of adipocyte differentiation as in the case of TZD treatment of diabetic animals. Rather, the adipocyte enlargement decreased significantly due to the deletion of one PPARγ allele and consequent protein loss [Kubota et al., 1999]. In fact the insulin-sensitizing effect in these animals is hypothesized to be due to the formation of smaller and more insulin-sensitive adipocytes as a result of the decreased PPARγ activity during development [Hallakou et al., 1997; Okuno et al., 1998]. Human genetic studies offered more insight into the puzzling relationship between PPARγ activity in the adipose tissue and insulin sensitivity. The activity of PPARγ2, the adipose tissue-specific subtype, is decreased in subjects with a Pro12Ala (substitution of Ala for Pro at position 12 of PPARγ2) mutation, which is only within the PPARγ2-specific amino terminus [Deeb et al., 1998; Altshuler et al., 2000]. These subjects have lower body mass index and better insulin sensitivity than normal controls [Deeb et al., 1998; Altshuler et al., 2000]. It is possible that these subjects may have smaller and more insulin-sensitive adipocytes as a result of decreased PPARγ2 activity in their fat tissues. These data together suggest reduced activity of either PPARγ1 or PPARγ2 can increase insulin sensitivity, possibly through formation of smaller but metabolically more active adipocytes, which is a mechanism different from that of PPARγ activation. The important role of PPARγ2 in adipogenesis is revealed in a recent study where PPARγ-null 3T3-L1 cells were used to assess the adipogenic effects of both PPARγ isoforms [Ren et al., 2002]. The study showed that only PPARγ2 was capable of inducing adipocyte differentiation in the PPARγ-null 3T3-L1 cells, suggesting that PPARγ2 is unique for adipogenesis [Ren et al., 2002].

SELECTIVITY OF PPARγ ACTIVATION: SPPARMS?

The X-ray crystal structure of the human PPARγ ligand-binding domain (LBD) reveals a large binding pocket, which may explain the diversity of ligands for this nuclear receptor [Nolte et al., 1998]. Novel PPARγ compounds chemically distinct from TZDs have been discovered such as the tyrosine-based GW7845 [Cobb et al., 1998; Suh et al., 1999], the indole GW0207 [Henke et al., 1999], and the isoxazolidinedione JTT-501 [Shibata et al., 1999]. In addition, structural features important for co-activator recruitment by the liganded receptor were also identified [Nolte et al., 1998]. The ligand-dependent activation of PPARγ occurs after the conformational change caused by ligand (or agonist) binding followed by the recruitment of co-activators. How many co-activators are involved in PPARγ activation and how they are recruited in different tissues is still unclear. One interesting phenomenon is that the rank order of binding affinity of TZDs for PPARγ is not correlated with the order of potency of their antidiabetic effects in vivo, suggesting that the in vivo activation of PPARγ by TZDs may involve other factors. This may lie in the abilities of the different TZDs to recruit

co-activators and assemble transcriptional complexes that transactivate different sets of target genes [Olefsky and Saltiel, 2000]. The differential abilities of agonists to recruit co-activators are characterized by the concepts of full and partial agonists, as suggested by Miles et al. and others [Miles et al., 2000; Olefsky and Saltiel, 2000; Rangwala and Lazar, 2002]. A full agonist refers to a compound that upon binding to PPARγ, co-activators in target tissues or cells are fully recruited, which facilitates the transcriptional activation to the maximal degree. Whereas a partial agonist refers to a compound that is only capable of recruiting a subset of co-activators and, therefore, only partially activates the nuclear receptor. It should be noted that a partial agonist is also automatically a partial antagonist that inhibits the activation of the nuclear receptor by a full agonist [Camp et al., 2000]. Since the concept is based on the ability of a ligand to recruit co-activators, the abundance of co-activators in a tissue is a major factor in defining the extent of a ligand's agonism. An additional factor is the kinetics of co-activator recruitment that may affect the assembly of transcriptional complexes [Olefsky and Saltiel, 2000]. The agonist and antagonist properties depend on the context of tissue and target gene [Olefsky and Saltiel, 2000]. A ligand can be a full agonist in one tissue where there are sufficient co-activators that are recruited but a partial agonist in selective tissues where there are insufficient recruited co-activators. For example, troglitazone can behave both as full and partial agonist, depending upon the type of tissue or cells for action [Camp et al., 2000]. In muscle and kidney cells, troglitazone behaves as partial agonist for PPARγ activation and antagonizes rosiglitazone-induced PPARγ transcriptional activity. However, in adipocytes, troglitazone acts as full agonist and induces PPARγ activation to a maximal extent similar to that by rosiglitazone [Camp et al., 2000]. This may explain the discrepancy between the rank order of PPARγ activation by TZDs and their antidiabetic activities. This finding also raised an interesting possibility that troglitazone may activate PPARγ differentially in different tissues depending on the relative levels of available co-activators in the target tissue. The recruitment of co-activators may also depend on the unique structural conformation of the liganded PPARγ caused by the bound agonist. This concept, similar to that of selective estrogen receptor (ER) modulators (SERMs) [Shang et al., 2000], was named SPPARMs (selective PPAR modulators) [Miles et al., 2000; Olefsky and Saltiel, 2000; Rangwala and Lazar, 2002]. It suggests that SPPARMs could be designed and developed to eliminate unwanted side effects in specific tissues. Recent advances in search of additional classes of PPARγ modulators have resulted in the discovery of novel compounds with differential potency and selectivity. The data collected with these new classes of partial and full agonists further prove the concept of SPPARMs. FMOC-L-Leucine (F-L-Leu), a chemically distinct PPARγ full agonist, improves insulin sensitivity in diabetic ob/ob mice and yet has a weak adipogenic activity [Rocchi et al., 2001]. This finding suggests that the insulin-sensitizing activity and the adipogenic activity of PPARγ activation can be differentiated by selective agonists. In contrast to the "1 ligand/1 receptor" paradigm that TZDs follow, F-L-Leu binds to PPARγ in a 2:1 ratio. The conformational change upon F-L-Leu binding is unique in that the liganded PPARγ recruits co-activators in a different pattern [Rocchi et al., 2001]. The data in this study suggests that the pattern of co-activators recruitment might determine the selectivity of SPPARMs. This notion is supported by a study with a novel synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), a PPARγ partial agonist [Wang et al., 2000]. Compared with the full agonist rosiglitazone, CDDO has a weaker ability to recruit the coactivator CREB-binding protein to PPARγ [Wang et al., 2000].

ROLE OF PPARγ IN THE TREATMENT OF ATHEROSLCEROSIS

Although atherosclerosis has been a major cause of death among type II diabetic patients and TZDs improve the lipid profiles in these patients, the role of PPARγ activation in atherosclerosis is not clear. The marginal correction of dyslipidemia after TZD treatment may indirectly contribute to the decreased risk of atherosclerosis in these patients. However, it appears that PPARγ may have direct effect on the formation and progression of atherosclerotic lesions. PPARγ is prominently expressed in the activated monocytes and macrophages, including foams cells in the atherosclerotic lesions [Jiang et al., 1998; Ricote et al., 1998a,b]. Initial

observations from studies aimed at determining the role of PPARγ in the pathogenesis of atherosclerosis generated conflicting results. Activation of PPARγ increased the expression of CD36, a class B scavenger receptor implicated in the uptake of oxidized low density lipoprotein (oxLDL) and thought to be critical player in foam cell formation [Feng et al., 2000]. Additionally, CD36 is highly expressed in lipid-laden macrophages in human atherosclerotic plaques [Nakata et al., 1999]. Moreover, HODEs, components within oxLDL, have been identified as PPARγ ligands [Kliewer and Wilson, 1998]. Thus, it has been proposed that the uptake of oxLDL by the macrophages would further induce oxLDL uptake by activating PPARγ and enhancing CD36 expression [Ricote et al., 1998a; Nakata et al., 1999; Feng et al., 2000]. This feed-forward loop, namely, the "PPARγ cycle," would promote lipid accumulation in macrophage cells and lead to the formation of atherosclerotic foam cells. However, based on these findings, PPARγ would be expected to be pro-atherogenic, a role inconsistent with observations of TZD effects seen in the clinic. This contradiction can in part be explained by the activity of PPARγ in other cell types within lesions [Iijima et al., 1998]. Troglitazone suppresses migration and proliferation of vascular smooth muscle cells (VSMC) induced by high glucose [Graf et al., 1997; Yasumari et al., 1997] or growth factors [Law et al., 1996]. Troglitazone also inhibited intima formation following balloon injury in Zucker fatty rats [Shinohara et al., 1998]. Further, PPARγ activation inhibited leukocyte–endothelial cell interaction, an inflammatory response critical for the formation of atherolsclerotic plaques [Jackson et al., 1999]. Consistent with this finding, PPARγ inhibits the expression of vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1) in activated endothelial cells [Pasceri et al., 2000]. This would significantly reduce the homing of monocyte and macrophage cells to atherosclerotic plaques. Taken together, these observations suggest that PPARγ activation by TZDs appears to be anti-atherogenic. This notion was further supported by evidence from studies with animal models, where several independent groups assessed the effect of TZDs in either low-density lipoprotein receptor (LDLR) or apolipoprotein E (apoE) knockout mice [Li et al., 2000; Chen et al., 2001; Collins et al., 2001; Glass, 2001], which are prone to atherosclerosis. These investigators found that troglitazone and rosiglitazone strongly inhibited atherosclerosis in these animals despite increased expression of CD36. In addition, GW7845, a tyrosine-based class of PPARγ agonist, had a similar effect [Li et al., 2000], suggesting PPARγ activation is likely the underlying mechanism.

Given the fact that PPARγ may be anti-atherogenic, how does the induction of CD36 expression by PPARγ fit in? Since CD36 is a scavenger receptor for oxLDL uptake, activation of PPARγ is expected to result in the accumulation of lipids in macrophage cells and foam cell formation. However, careful HPLC analysis of the lipid content induced by PPARγ activation within the macrophage demonstrated that the lipids that accumulated in TZD treated macrophages were composed mainly of triglycerides. In fact, troglitazone treatment had little or no effect on total cholesterol or cholesterol ester accumulation in macrophage cells treated with oxLDL [Moore et al., 2001]. Moreover, CD36 is not the only scavenger receptor that is mediated by PPARγ. The expression of scavenger receptor class A (SRA), another scavenger receptor utilized in the uptake of modified lipids, is suppressed by PPARγ [Moore et al., 2001]. In addition, the expression of ABCA1, a transporter that mediates cholesterol efflux from macrophage cells, is induced by PPARγ activation [Chinetti et al., 2001]. The anti-atherogenic effect of PPARγ activation is a result of its coordinated effects on cholesterol influx and efflux. The suppression of SRA expression and induction of ABCA1 expression countered the induction of CD36 expression [Lazar, 2001].

As a result, cholesterol accumulation in macrophage cells is not changed after PPARγ activation by TZDs [Lazar, 2001]. In fact, the in vivo data with LDLR [Li et al., 2000; Collins et al., 2001] or apoE knockout mice [Chen et al., 2001] suggest that cholesterol accumulation in macrophages might be prevented by TZD-induced PPARγ activation (Fig. 2). The anti-inflammatory effects of PPARγ agonists might also contribute to their anti-atherogenic activities (Fig. 2). The activation of PPARγ in human CD-positive T cells inhibits the expression of proinflammatory cytokines such as IFNγ [Marx et al., 2002]. Further, PPARγ agonists inhibit inflammatory cytokine production [Jiang et al.,

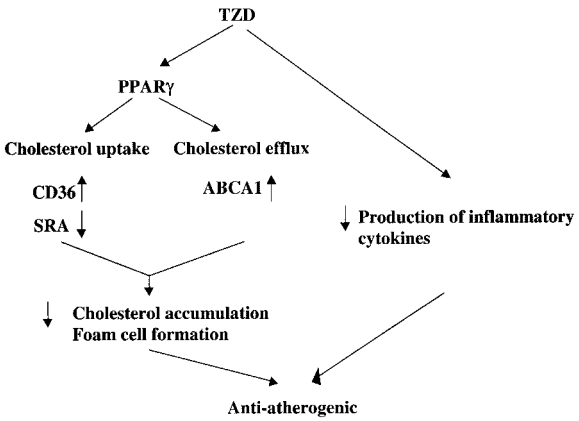

Fig. 2. Proposed mechanisms of action for TZDs' anti-atherogenic effect.

1998] and macrophage activation [Ricote et al., 1998b].

CONCLUSION

The identification of TZDs as synthetic PPARγ ligands has greatly advanced our understanding of the mechanism of action, the selectivity, and the biological importance of PPARγ. In the meantime, the discovery and synthesis of novel classes of PPARγ agonists has provided important tools to delineate receptor activation, selective recruitment of co-activators, and tissue selectivity. In support of the SPPARMs concept, the initial differentiation of insulin sensitizing activity and adipogenesis with a full agonist has been reported. Most importantly, while the underlying mechanism of PPARγ in the treatment of type II diabetes is being investigated, the emerging role of PPARγ in atherogenesis has been revealed by findings in atherosclerotic animal models as well as molecular regulations of genes involved in cholesterol fluxes and inflammatory responses. The target genes for PPARγ in its anti-atherogenic effects are those involved in cholesterol fluxes and inflammatory responses. The role of PPARγ in atherosclerosis provides an exciting opportunity for potential use of TZDs or other PPARγ agonists in the treatment of atherosclerosis in general population.

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