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Commentary

Toxicological consequences of altered peroxisome proliferator-activated receptor γ (PPARγ) expression in the liver: insights from models of obesity and type 2 diabetes

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Abstract

The pivotal role of peroxisome proliferator-activated receptor γ (PPARγ) in the liver, although important for the regulation of genes involved in glucose and lipid metabolism, has generally not been fully appreciated. This may be due to the fact that PPARγ, in contrast to PPARα or PPARδ, is not abundantly expressed in liver under normal conditions. However, recent findings have revealed that in several murine models of obesity and type 2 diabetes mellitus (T2DM), PPARγ mRNA and receptor protein are highly up-regulated in the liver, and that the receptor causes increased transcriptional activity as demonstrated by the activation of PPARγ-responsive genes in the liver. Prolonged treatment of obese and diabetic mice, but not of lean control mice, with the selective PPARγ ligands and activators, thiazolidinediones (TZDs), including troglitazone, rosiglitazone, or pioglitazone, has resulted in the development of severe hepatic centrilobular steatosis. In contrast to these effects in hepatocytes, TZD-mediated effects on Kupffer cells (down-regulation of proinflammatory cytokines) seem to be PPARγ-independent. In view of the findings that sustained hepatic steatosis can lead to steatohepatitis and/or fibrosis and that troglitazone (but not the other TZDs) has been associated with rare but serious hepatotoxicity in patients, further insight into PPARγ-mediated versus non-PPARγ-mediated effects of TZDs is desirable. It is concluded that liver-specific effects associated with TZD antidiabetics may become relevant under conditions of selective PPARγ up-regulation in the liver. Therefore, receptor expression in human liver tissue of obese and T2DM patients should deserve increased consideration in the future.

Keywords: Peroxisome proliferator-activated receptor γ (PPARγ); Thiazolidinediones; Troglitazone; Liver; Hepatic steatosis; Type 2 diabetes mellitus (T2DM)

1. Introduction

In the past years, the role of the nuclear hormone receptor and transcription factor family, the PPARs, has gained renewed appreciation for two major reasons. First, in-depth molecular analysis of the novel isoforms, PPARγ and its variants, has given us major insight into the gene structure, regulation, and differential expression of this receptor in various tissues. Second, with the advent of a novel class of antidiabetic drugs, the TZDs, and with the awareness that these drugs exhibit high-affinity binding and selectivity for PPARγ, novel pathways for the treatment of T2DM, based on a molecular rationale, became apparent.

PPARγ has generally been described to exhibit an adipose-selective expression. It has, therefore, been implicated in lipid metabolism and in the modulation of adipose tissue [1,2]. However, the extensive range of effects that this receptor can exert in extra-adipose tissues, including hepatic parenchymal cells, has only recently been appreciated. Reasons for having underestimated the role of PPARγ in liver include: (i) the low basal expression of PPARγ in liver, (ii) focus on expression and regulation of the other PPAR form, PPARα, which is abundant in liver and whose activation by ligands has been associated with a number of hepatic effects, and (iii) a lack of reports on hepatic adverse effects in

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; NEFA, non-esterified fatty acid; T2DM, type 2 (non-insulin-dependent) diabetes mellitus; LPS, lipopolysaccharide; NSAIDs, nonsteroidal anti-inflammatory drugs; IL, interleukin; TNF α, tumor necrosis factor-α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; and TZD, thiazolidinedione.

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small laboratory animals treated with PPARγ agonists. Only recently was it found in experimental models that hepatic PPARγ can be up-regulated by nutrition and natural ligands, such as fatty acids or fatty acid metabolites. The functional consequences of up-regulated PPARγ expression in the liver, which can thus become a target for PPARγ ligands under these conditions, has not been adequately explored. The aim of this commentary is to critically address the possibility of a significant interaction of PPARγ agonists with up-regulated PPARγ in the liver and to discuss possible downstream consequences of PPARγ activation in several cell types of the liver.

We believe that a critical evaluation and reassessment of hepatic effects of PPARγ ligands are both timely and crucial. This is because one of the first generation TZDs and PPARγ ligands, troglitazone, has been associated with hepatic toxicity in T2DM patients, while two other TZDs (pioglitazone and rosiglitazone), given at much lower doses and metabolized by other pathways, have not been reported to cause liver injury. The mechanisms underlying this rare but severe drug adverse reaction associated with troglitazone are not clear. However, it is important to determine whether the hepatic liability is related to the compound-specific metabolic bioactivation and disposition or, alternatively, whether there is a class effect component, related to abnormal expression of PPARγ in the liver in a subset of susceptible patients, and which would contribute to these hepatic effects induced by selective interaction of some, but not all, TZD and non-TZD agonists of PPARγ.

It is concluded from this critical review that exposure to TZDs of obese and diabetic laboratory rodents, which feature highly up-regulated PPARγ in the liver, can indeed have pathophysiological consequences. Currently, it is not known whether similar mechanisms might be involved in patients, and the available information on the regulation of PPARγ in human liver, as opposed to that in other organs, is scanty.

2. PPARγ

2.1. PPARγ1 and γ2 as a family of "adipocyte-selective" transcription factors regulating lipid homeostasis

Enormous progress has been made in the past years on elucidating the molecular genetics of the nuclear hormone receptor and transcription factor PPARγ. These efforts were mainly driven by the increasing awareness of the pivotal role of the receptor as a therapeutic target in treating insulin resistance and T2DM.

Mammalian PPARγ is encoded by a single gene, which features a highly conserved structure in mice, rats, and humans [3–5]. At least three transcript forms (PPARγ1, γ2, and γ3) arise from alternative promoter usage and by alternative splicing, while at the protein level two isoforms, PPARγ1 and γ2, have been identified. Among these, PPARγ1 is translated from PPARγ1 mRNA, but also may arise from γ3 transcripts by independent promoter usage in human adipose tissue and colon epithelium [6]. PPARγ2, translated from PPARγ2 mRNA, contains an additional 30 amino acids at the N-terminal end [3,4]. The differential function of these splice variants is not known, but their distinct tissue distribution suggests that they may have differential physiological roles [7].

PPARγ regulates the transcription of a number of genes involved in glucose and lipid homeostasis and maintaining normal insulin responsiveness. This complex regulation, which is incompletely understood, involves the formation of heterodimers of PPARγ with the retinoic X receptor, which binds to PPAR response elements within the promoter region of target genes, and which is coupled with a co-repressor and a coactivator complex [8]. Many of the target genes are directly involved in lipogenic pathways in adipocytes [9] and include lipoprotein lipase [10], adipocyte fatty acid binding protein aP2 [2], acyl-CoA synthase [11], and fatty acid transport proteins [12].

In the search for the natural ligand(s) for PPARγ, a prostaglandin metabolite, 15d-PGJ_2, was first identified. In addition, a number of polyunsaturated fatty acids, including linoleic acid, were subsequently found to be ligands of PPARγ [13–15]. Because of the relatively low receptor affinity of these natural ligands, and in view of the relatively small concentrations of "free" (non-bound) fatty acids in cells, their biological role has, however, been questioned [16]. Nevertheless, PPARγ has generally been considered a "fatty acid sensor" that regulates the storage of fatty acids, predominantly in the adipose tissue.

Ligand binding causes a conformational change in PPARγ [17]. Thereby, the receptor is converted into an activated form that facilitates the recruitment of coactivators including p300 and steroid receptor coactivator (SRC-1). The function of these coactivators is not entirely clear, but it has been suggested that they link the regulatory signals of the receptor with the transcriptional machinery. This could explain why different (synthetic and natural) ligands exert differential responses upon binding and activation of the receptor [18].

Because PPARγ levels (predominantly PPARγ2) in fat tissue are 10–100 times higher than those in other organs, PPARγ has long been considered an "adipose-selective" nuclear receptor. However, other tissues expressing the receptor, although less abundantly, have recently gained increased attention.

2.2. PPARγ1 expression in extra-adipose tissues

High expression levels of PPARγ were found in the colon epithelium, where the nuclear receptor has been implicated in regulating apoptosis and where it could play a role in colonic cancer [19]. Similarly, the kidney and small intestine exhibit intermediate levels of PPARγ [4,20]. In all

Table 1
Increased hepatic expression of PPARγ transcripts in various models of obesity and insulin resistance

Animal model	Phenotype	Change in basal expression of hepatic PPARγ1 or γ2 mRNA (vs wild-type control)	Reference
PPARα −/− mice	Knockout, PPARα-deficient, obese	PPARγ2 ↑ ~1.5 × in whole liver, ~10 × in fat-loaded hepatocytes (vs C57BL/6 mice)	[32]
UCP-DTA mice (on high fat diet)	Transgenic, no brown adipose tissue	PPARγ2 ↑ (vs FVB/N mice)	[22]
aP2/DTA mice	Transgenic, no brown and white adipose tissue, hyperleptinemic, insulin resistant	PPARγ[a] ↑ 1.3 × (vs wild-type)	[25]
A-ZIP/F1 mice	Transgenic, no white adipose tissue	PPARγ[a] ↑ 3–17 × (vs FVB/N mice)	[24]
ob/ob Mice	Leptin-deficient	PPARγ2 ↑ (vs +/? lean controls)	[26]
		PPARγ1 ↑ 6 ×	[28]
		PPARγ2 ↑ 13 × (vs C57BL/6 mice)	[28]
KKA[y] mice	Overexpression of yellow Agouti gene by mutation of Raly gene, obese, hyperleptinemic	PPARγ1 ↑ 6 × (vs C57BL/6 mice)	[28]
		PPARγ2 ↑ 2 ×	
db/db Mice	Leptin receptor-deficient	PPARγ[a] ↑ 7–9 × (vs C57BL/6)	[27]
5-HT2cR mutant mice	Serotonin 5-HT2c receptor mutant mice, hyperleptinemic, late onset obesity	PPARγ[a] ↑ 2 × (vs C57BL/6)	[27]

[a]Isoform not specified.

of these human extra-adipose tissues that have been analyzed, PPARγ1 was the predominant isoform [4].

In a number of other organs, including skeletal muscle and liver, PPARγ is expressed at only very low basal levels. This may be one of the reasons why PPARγ has been considered to be less important in these organs. Increasing evidence suggests, however, that PPARγ plays a significant role in extra-adipose tissues too, for example, in regulating fatty acid metabolism in skeletal muscle [21].

Perhaps another reason for having underestimated the role of PPARγ in extra-adipose tissues was the fact that most of the quantitative data on PPARγ expression and tissue distribution were derived from the analysis of normal rodents or healthy individuals. Because PPARγ is tightly regulated by a number of epigenetic factors, the relative expression of PPARγ can thus quickly and dramatically change under pathophysiological conditions.

3. Regulation of PPARγ in liver

3.1. Up-regulation of hepatic PPARγ1 and/or γ2 in murine models of obesity and T2DM

PPARγ is normally expressed in both human and murine liver at only 10–30% of the levels in adipose tissue [2,4]. What was detected under these basal conditions was primarily PPARγ1, while γ2 was present in the liver in trace amounts only [4,20].

However, it is known that obesity and nutrition can up-regulate PPARγ expression in the liver [22,23]. The mechanism underlying this induction is not clear, but it has been suggested that during increased energy availability, a circulating factor may stimulate hepatic PPARγ transcription [24]. Interestingly, in a number of murine models of obesity and T2DM, all of which feature different etiology but result in a similar phenotype, it was found that the hepatic levels of both PPARγ transcript and protein expression were highly increased (Table 1). For example, in different transgenic mouse strains, which are deficient in white or brown (or both) adipose tissue and all of which exhibit lipoatrophic diabetes, obesity, and insulin resistance, the basal PPARγ levels in the liver were increased several-fold [22,24,25]. In other mutant models of obesity and T2DM, in which leptin or the leptin receptor was disrupted, the hepatic expression of PPARγ mRNA and protein was similarly up-regulated [26–28]. It should be noted, however, that the numbers (fold up-regulation) should be taken with caution as the basal levels in the corresponding wild-type strains are very low, making exact calculations difficult.

Depending upon the specific mouse model, the two isoforms were differentially up-regulated. For example, we have demonstrated recently that in ob/ob mice the PPARγ2 form is primarily induced, while in KKA[y] mice there is a selective PPARγ1 induction, both at the mRNA and protein level [28]. The reasons for this differential induction and possible downstream biological consequences are not known.

3.2. Cell-type-selective expression of PPARγ in liver

Although we have gained more insight into the cell-type specific expression and intralobular distribution of PPARγ in mammalian liver in the past years, our understanding of the cell-selective up-regulation of PPARγ is still incomplete. This has two major reasons: first, most quantitative data on PPARγ expression are generated from whole-organ homogenates, and second, localizing PPARγ expression in tissue sections by immunohistochemistry or *in situ* polymerase chain reaction (PCR) has remained difficult. It would be highly desirable, however, to have more funda-

mental knowledge about the downstream biological consequences associated with the different cell types in the liver.

What is known is that PPARγ is not only expressed in Kupffer cells (the resident hepatic macrophages) [29], as would be expected from the well-known findings that PPARγ is abundant in the monocyte/macrophage lineage [30,31], but also in hepatic parenchymal cells. For example, the presence of both PPARγ transcripts and immunoreactive protein was demonstrated in normal hepatocytes (>99% pure) isolated from rat liver [29]. Furthermore, isolated hepatocytes from PPARα−/− mice, which develop late-onset obesity [32], exhibited greatly increased PPARγ2 expression that was approximately 10-fold higher than that in hepatocytes from normal (PPARα+/+) control mice. Thus, there is experimental evidence that the overexpression of PPARγ, as determined in whole liver homogenates, is due, at least in part, from increased levels of the receptor in hepatocytes. In addition to hepatocytes and Kupffer cells, isolated hepatic stellate cells also exhibit biological responses to PPARγ agonists [33], implicating a role of these cells in PPARγ-mediated signaling in the liver. Finally, little is known about PPARγ expression in hepatic endothelial cells; the receptor has been demonstrated, however, to be present in extrahepatic endothelial cells [34].

4. Hepatic effects of drugs that bind to and activate PPARγ

4.1. TZD-induced differential gene expression in normal liver and in liver of obese and diabetic rodents

TZDs (glitazones), used to treat insulin resistance and T2DM, were the first class of compounds found to specifically bind to and activate PPARγ [reviewed in Refs. 16 and 35–39]. Among these TZDs, three drugs have been therapeutically used: troglitazone (which has been withdrawn from the market), pioglitazone, and rosiglitazone. Their affinity for PPARγ differs widely; for example, activation of human PPARγ1 in transactivation assays ranked the three TZDs as follows: rosiglitazone > pioglitazone ≥ troglitazone, with rosiglitazone featuring an affinity that was approximately 100-fold higher than that for troglitazone [40–42]. Furthermore, different TZDs, although binding to the same receptor, can have multiple downstream biological effects. For example, troglitazone and rosiglitazone can differentially down- or up-regulate specific proteins [41].

In view of the highly up-regulated expression of PPARγ in the liver of obese and diabetic animals, one would predict that the effect on the liver induced by TZDs would be different in these obese animals as compared with those in normal animals. Indeed, treatment of ob/ob mice with rosiglitazone caused a compound-selective effect, manifested by significant up-regulation of >15 proteins (as revealed by two-dimensional gel electrophoresis analysis) only in the obese mice, but not in their lean controls [26]. Because the obese mice exhibited increased PPARγ2 expression, it is conceivable that this condition rendered the mice susceptible to the effects of the drug.

Because PPARγ regulates lipid homeostasis, specific effects on lipid metabolism would be expected to occur in mice overexpressing the receptor in the liver. Indeed, a recent report demonstrated that troglitazone treatment of ob/ob mice increased the expression of adipocyte fatty acid-binding protein (aP2) and fatty acid translocase (FAT/CD36) in the liver, both of which are expressed at very low levels or are not detectable at all in normal liver [27].

4.2. Disruption of lipid homeostasis in liver and development of hepatic steatosis by TZD activators of PPARγ

The biological effects of PPARγ activators in the liver in which the receptor is highly up-regulated can have dramatic consequences on lipid homeostasis. For example, KKA^y mice given pioglitazone increased hepatic diacylglycerol levels 2- to 3-fold [43]. Diacylglycerol is an intermediate for the synthesis of both triglycerides and phospholipids. Chronic treatment of KKA^y mice with pioglitazone also resulted in the development of markedly distended hepatocytes with evidence of severe lipid generation [44]. We recently demonstrated that such KKA^y mice, but not lean control mice, treated for 4 weeks with two other TZDs, troglitazone or rosiglitazone, developed marked centrilobular steatosis [28]. The fatty infiltration, which was particularly severe with rosiglitazone, was not accompanied by clinical-chemical signs of liver injury. These findings were confirmed in another animal model of obesity and T2DM, the db/db mouse, where 4-week oral treatment with troglitazone, pioglitazone, or rosiglitazone resulted in a slight to moderate fatty change in hepatocytes [45].

Interestingly, in a recent study using ob/ob mice, troglitazone treatment was not associated with an increase in hepatic lipids (in fact, a slight decrease in total lipids was observed) [27]. One possible reason for these apparently paradoxical results could be that the livers of untreated ob/ob mice are already highly steatotic before treatment with TZDs is initiated. Alternatively, it is not known whether the response towards a PPARγ2-selective up-regulation, as it is occurring in ob/ob mice [28], is different from a hepatic response with PPARγ1 being selectively induced.

The pathogenesis of TZD-mediated hepatic steatosis is not known, but the array of hepatic functional changes in these models allows for establishing a unifying hypothesis (Fig. 1). As there are increased fluxes of NEFAs from the adipose tissue to the liver in obese animals, hepatocytes are continuously exposed to high NEFA levels, at least initially, before the plasma NEFA levels begin to gradually decrease during TZD treatment. In hepatocytes with high expression levels of PPARγ, a number of concerted pathways will

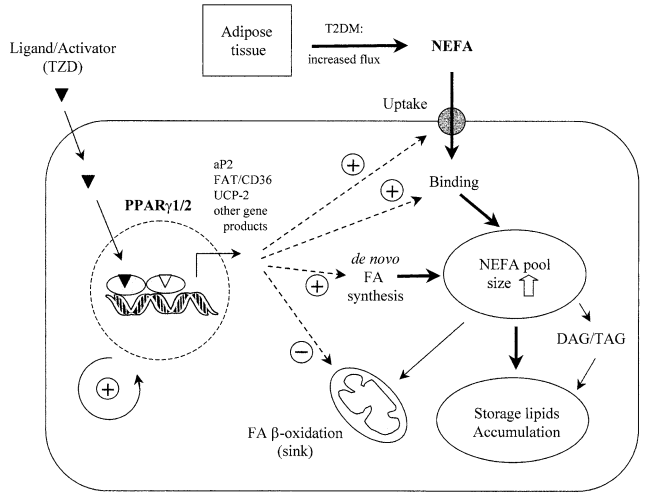

Fig. 1. Putative PPARγ-mediated pathways of altered fatty acid metabolism and disposition in hepatocytes of obese and diabetic mice exposed to TZDs. High expression levels of PPARγ are associated with the induction of PPARγ-responsive genes, resulting in enhanced uptake and intracellular binding of NEFAs and increased fatty acid (FA) biosynthesis. In contrast, mitochondrial FA β-oxidation is decreased. This leads to a net increase in the hepatocellular NEFA pool and to accumulation of storage lipids. Key: (+) up-regulation; and (−) down-regulation.

further increase the net NEFA levels. For example, *de novo* synthesis of fatty acids is increased because TZDs augment the insulin-stimulated hepatic conversion of glucose into fatty acids [46]. Furthermore, TZDs induce the hepatic expression of PPARγ-responsive genes involved in fatty acid uptake and binding, including the long-chain fatty acid-binding protein aP2, and the fatty acid transporter FAT/CD36 [27]. Finally, and importantly, hepatocellular fatty acid degradation is attenuated by TZDs because the drugs inhibit long-chain fatty acyl β-oxidation, which normally is a quantitatively important sink for NEFAs in hepatocytes [47–49]. Sustained inhibition of mitochondrial β-oxidation can lead to microvesicular steatosis [50].

It cannot be excluded that, besides PPARγ, other PPARγ-independent pathways are also activated and are involved in the generation of hepatic steatosis. For example, sterol regulatory element-binding proteins (SREBPs), a family of transcription factors that not only regulate cholesterol production but also fatty acid synthesis in the liver, could play a role. Indeed, ob/ob mice exhibited increased expression of nuclear SREBP-1 in liver, along with increases in mRNAs for multiple lipogenic genes, including those for fatty acid biosynthesis [51].

If the increased hepatic PPARγ expression is causally involved in the development of these pathological changes, then the regulatory effects of the TZDs on the expression of the receptor itself would further modulate these effects. Indeed, high doses of troglitazone increased the expression of PPARγ mRNA and protein several-fold in rats [52]. Similarly, rosiglitazone treatment caused a slight increase of PPARγ expression in diabetic A-ZIP/F1 mice [24]. Thus, TZDs could further sensitize the liver for the PPARγ-mediated effects. It has even been suggested that this positive feedback loop might gradually elevate hepatic PPARγ levels in patients during TZD treatment [16].

The consequences of sustained hepatic lipid accumulation, if its degree has become severe, could result in a number of secondary adverse effects. For example, the "two-hit" scenario implicated in fatty degeneration of the liver could become active [53]. This model implies that the first hit (sustained lipid accumulation), which would also facilitate storage of the lipophilic drug itself in the liver cell, will be followed by the second hit, due to the presence of lipid peroxidation products including protein-reactive aldehydes, which are invariably seen in fatty liver. Fatty livers are also more susceptible to ischemia/reperfusion injury [54] or LPS-mediated liver injury [55,56]. Finally, steatotic livers might also see some disruption of energy homeostasis, because one of the genes that is induced by TZDs in obese mice is the uncoupling protein UCP-2 [27]. Expression of UCP-2 in mitochondria leads to increased respiration and to a gradual depletion of ATP due to the uncoupling of oxidative phosphorylation from electron transport, thus enhancing the vulnerability of hepatocytes to necrosis-inducing stimuli [57].

4.3. NSAIDs as activators of PPARγ

A number of non-TZD drugs are also able to bind to PPARγ. For example, NSAIDs, including indomethacin, fenoprofen, ibuprofen, and flufenamic acid, have been shown to activate PPARγ [58]. These compounds were able to induce the differentiation of murine fibroblasts to mature adipocytes. Not only was there increased lipogenesis and fat accumulation in these cells, but also a direct induction of the expression of the adipocyte-specific fatty acid binding protein, aP2, whose expression is directly regulated by PPARγ. One could speculate that these effects were indirect, due to the NSAID-mediated inhibition of cyclooxygenase and, hence, accumulation of arachidonic acid or other precursors and activators of PPARγ. However, not all cyclooxygenase inhibitors caused adipocyte differentiation. Thus, some of the cellular effects of NSAIDs may not be mediated solely through the inhibition of prostaglandin synthesis but also by activation of PPARγ.

These findings could help to explain, in part, the well-known effects of some NSAIDs (in particular the 2-arylpropionic acids) in causing, in rare cases, hepatic microvesicular steatosis. Mitochondrial inhibition of β-oxidation caused by sequestration of CoA, thus preventing import of CoA-dependent carnitine-mediated long-chain fatty acids into the mitochondrial matrix, has been invoked to account for these effects. However, the concentrations needed to elicit these mitochondrial effects are equally high (micromolar range) as those needed to activate PPARγ. Therefore, activation of PPARγ-mediated pathways leading to down-regulation of mitochondrial β-oxidation might offer a plausible comple-

mentary mechanism for the development of fatty liver in susceptible individuals. However, our knowledge about the possible effects of NSAIDs on PPARγ in the liver is still fragmentary.

4.4. Induction of apoptosis by PPARγ ligands

PPARγ ligands have been implicated in inducing apoptosis in a number of cell types. For example, rosiglitazone (at low concentrations, in the range of its K_D value of 20 nM) was able to increase the number of TUNEL-positive cells and to increase activation of caspase-3 in differentiated macrophages [59]. Similarly, TZDs triggered apoptosis in cultured astrocytes [60] or in B lymphocytes [61] via PPARγ. The mechanism underlying the induction of apoptosis is not yet clear, but evidence suggests that TZDs could interfere with the anti-apoptotic NF-κB signaling pathway [59,62–64]. It has not been determined whether a similar NF-κB inhibition might be responsible for the observed TRAIL-induced pro-apoptotic effects of TZDs, which enhance apoptosis in tumor cells [65]. To date, no reports are available on ligand-induced apoptosis in liver with high PPARγ expression levels.

4.5. Down-regulation of PPARγ in activated stellate cells

Rat hepatic stellate cells express PPARγ1, but not PPARγ2 [66]. It is not clear, however, whether stellate cell PPARγ expression might be dysregulated in obesity and diabetes. This would be important to know in view of the recently disclosed causal link between PPARγ and hepatic stellate cell activation [33,67]. Stellate cells proliferate following a chemical insult and migrate towards the damaged areas, where they start to produce collagen and other extracellular matrix components. They also release chemotactic factors and recruit inflammatory cells. One of the mediators of stellate cell activation is oxidative stress, in particular lipid peroxidation products [68]. Such toxic species might be generated in severe hepatic steatosis; in fact, hepatic steatosis is a well-known precursor lesion for the development of fibrosis [69].

It has been reported recently that activation of PPARγ by selective ligands inhibits some of these key functions normally executed by stellate cells [67]. Specifically, troglitazone or 15d-PGJ_2 inhibit cell proliferation, migration, and chemokine expression of cultured stellate cells [33]. The mechanism of this negative regulation is not known. Because PPARγ transcripts were no longer found in activated stellate cells and in fibrotic liver, which was experimentally induced by bile duct ligation [66], PPARγ expression has been inversely correlated with stellate cell activation. Thus, stellate cell function would be inhibited only during the initial phases of TZD exposure. Again, the effects of TZDs on stellate cells in the liver of obese and diabetic mice have not been reported.

4.6. Ligand-activated down-regulation of cytokine production in Kupffer cells—a PPARγ-independent effect?

Because PPARγ is expressed abundantly in macrophages, including Kupffer cells, it would be important to assess the functional consequences of increased expression of PPARγ on the resident macrophage pool in the liver.

One important macrophage function is the production of cytokines and other proinflammatory response mediators. *In vitro* studies have revealed that PPARγ agonists, including TZDs, can abrogate the production of a number of proinflammatory cytokines (TNFα, IL-6, IL-1) and inducible nitric oxide synthase in murine macrophages [64], isolated and activated human monocytes [63], activated human lymphocytes [70], or isolated rat Kupffer cells [71]. The mechanism of this marked inhibition of macrophage function has not been fully elucidated; hypotheses to explain these effects have included TZD-mediated stimulation of heat shock protein hsp70, which is associated with cytokine signaling [72], or inhibition of NF-κB and AP-1 activation [71,73,74].

However, not all PPARγ ligands are able to inhibit proinflammatory cytokine production. For example, a number of high-affinity TZD and non-TZD ligands did not decrease IL-6 or TNFα in cultured human monocytes or macrophages [75]. Therefore, it is doubtful whether the inhibition of macrophage function is mediated by PPARγ. It has been suggested that some of the ligands that do inhibit cytokine production may exert this effect via a PPARγ-independent mechanism [75].

To exclude *in vitro* artifacts, induced by the high TZD concentrations that are normally used for cell culture studies, we recently investigated Kupffer cell function in the liver in an *in vivo* model. Specifically, we demonstrated that troglitazone administered to mice significantly decreased TNFα and IL-6 mRNA and liver cell-associated TNFα protein in the liver [76]. Since hepatic transcript levels of IL-6 and TNFα are low under basal conditions, the mice were challenged with an acute dose of LPS, which caused a massive increase in proinflammatory cytokine production in vehicle controls. In troglitazone-pretreated mice, in contrast, TNFα and IL-6 production was inhibited almost completely.

Because this inhibitory effect of troglitazone was more prominent in the livers of obese and diabetic KKA^y mice than in lean control mice, it is tempting to conclude that PPARγ, which is highly up-regulated in the liver of these obese mice, might be involved. Yet the findings that treatment of the same mouse strain with rosiglitazone did not result in an inhibition of cytokine production fuels the hypothesis that this effect on macrophages may be mediated by a PPARγ-independent pathway [76]. Alternatively, the two TZDs may cause differential conformational changes of PPARγ upon binding and thus result in differential downstream effects.

These data were confirmed and extended in db/db mice

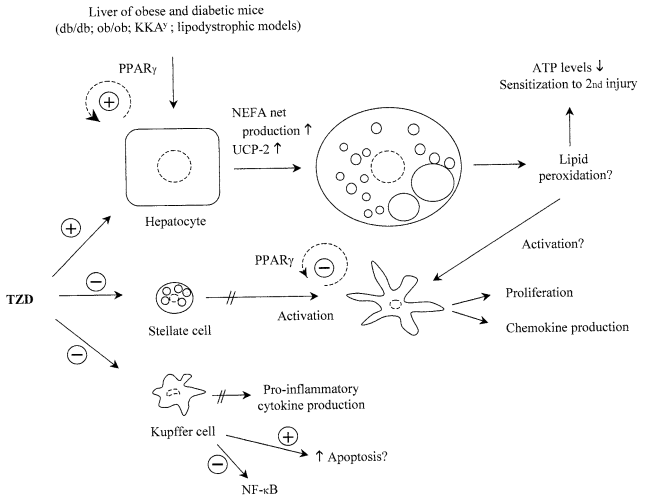

Fig. 2. Possible cell-specific consequences of TZD binding and PPARγ activation in the liver of obese and diabetic mice. Hepatocytes are transformed into lipid-laden cells, and hepatic microvesicular and macrovacuolar steatosis will ensue. In contrast, stellate cell activation is inhibited by PPARγ activation. Kupffer cell function is also decreased by TZDs via mechanisms that include PPARγ-independent pathways. Key: (+) stimulation; and (−) inhibition.

that were given AD 5075, a potent TZD, and did not respond with decreased production of IL-6 or soluble TNFα after an *in vivo* challenge with LPS [75]. That the down-regulation of some proinflammatory cytokines may be the result of multiple pathways was also convincingly demonstrated in a study using macrophages derived from embryonic stem cells that were homozygous for a null mutation in the PPARγ gene [77]. Stimulation with LPS in the presence of TZDs or 15d-PGJ_2 resulted in inhibition of TNFα and IL-6 secretion in both wild-type and PPARγ null macrophages, indicating that the effect of TZDs on cytokine production is PPARγ-independent.

5. Conclusions

The functional consequences of receptor activation by ligands (including TZDs) in liver with abnormally high levels of PPARγ, as it occurs in murine models of obesity and T2DM, can be several-fold (Fig. 2). The most important one seems to be induction of hepatic steatosis, with all its possible sequelae. It is not clear yet as to what cell types within the liver and to which sublobular areas these increased levels of PPARγ might be restricted, but it seems plausible that hepatocytes are subject to these regulatory changes. These target cells respond with a dramatic increase in fat accumulation upon exposure to TZDs, and TZDs can, in turn, further up-regulate PPARγ expression, thus initiating a vicious cycle. Effects of TZDs on the other cells in the liver are less clear; it seems, however, that the negative regulatory effects of TZDs on Kupffer cells may not be mediated by PPARγ, and that PPARγ ligands inhibit stellate cell activation.

An obvious question is whether the effects observed in obese and diabetic mice would also occur in humans. As obesity is a major risk factor in diabetes, it is possible that dysregulated PPARγ in liver could be present in a subset of patients. However, to date there is no information about altered hepatic PPARγ levels in obese and diabetic individuals, due in part to the poor availability of liver tissue from otherwise healthy people. In regard to adipose tissue, it is known that obesity and T2DM are not associated with changes in PPARγ mRNA [20]. However, adipose tissue PPARγ expression is not changed in obese and diabetic mice either [22], although their hepatic levels are increased, probably because in adipose tissue the induction has already reached maximal or submaximal levels due to the abundant presence of endogenous ligands including fatty acids [27].

Furthermore, there is no information available on shifts in the relative expression of PPARγ isoforms in the liver. Again, a human study involving biopsies from thirteen T2DM patients revealed that PPARγ1 expression in adipose tissue exhibited large individual variations [20], whereas, in another study, PPARγ2 mRNA was found to be increased in adipose tissue of obese patients [78]. Therefore, analysis of PPARγ expression levels in the liver of obese and T2DM patients should deserve increased commitment.

So far, there is no indication from pathological examinations of liver biopsies that diabetic patients who received troglitazone or another TZD exhibited a higher incidence of hepatic steatosis or steatohepatitis than patients who did not receive TZD treatment. Nevertheless, in some reported cases of troglitazone-associated hepatotoxicity, histopathological and ultrasound analysis revealed the presence of fatty liver [79,80]. Proof of causality is difficult as a large proportion of obese T2DM patients develop fatty livers independent of TZD treatment. However, this condition may lead to hepatic fibrosis and cirrhosis in a subset of individuals. Therefore, possible modulating effects of long-term TZD treatment should be investigated to define whether the steatogenic effect described in obese mice is restricted to this species. In contrast to mice, Otsuka Long-Evans Tokushima Fatty (OLETF) rats, another model of insulin resistance, did not develop fatty change of the liver after long-term administration of troglitazone [81].

In summary, there is ample evidence that PPARγ mRNA and protein are highly up-regulated in the liver of distinct murine models of obesity and T2DM. The most consistent downstream biological consequence upon receptor activation by TZDs in these mice is the transcriptional activation of selective genes involved in lipid metabolism and the development of hepatic steatosis. Although the effect of the various TZDs can evoke differential responses and gene activation, due to differential activation of PPARγ1 or γ2, differential cofactor recruitment, or differential conformational changes, possible alterations in PPARγ expression levels should be analyzed in humans with obesity and T2DM.

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