**Peroxisome proliferated-activated receptor γ ligand, Pioglitazone, does not prevent hepatic fibrosis in mice**

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**Abstract.** During hepatic fibrogenesis, quiescent hepatic stellate cells (HSCs) undergo phenotypic transformation into activated matrix-producing cells. This process is recapitulated in primary HSCs cultured on plastic. Based on studies in rats, peroxisome proliferator-activated receptor γ (PPARγ) has been suggested to play a key role in the control of HSC activation. Indeed, in rats, PPARγ expression is depleted in activated HSCs. PPARγ ligands inhibit HSC activation and prevent hepatic fibrosis in vivo. Here we evaluated the impact of PPARγ agonists on hepatic fibrogenesis in mice both in vitro and in vivo. Primary HSCs from Balb/C mice were cultured with PPARγ ligands Pioglitazone (PGZ) or 15-deoxy-Δ12,14 prostaglandin J1 (15d-PGJ1). PPARγ mRNA expression was stable during culture-activation of HSCs. However, PPARγ protein was only found in quiescent HSCs but not in fully activated cells. Exposure of HSCs to PPARγ agonists maintained the expression of PPARγ, and trans-activated this transcription factor as demonstrated by gelshift assay and by induction of CD36, a PPARγ-regulated gene. However, PPARγ ligands did not alter the induction of Collagen-I mRNA or α-smooth muscle actin (α-SMA) in cultured HSCs. To test the effect of PPARγ agonist PGZ in vivo, hepatic fibrosis was evaluated in Balb/C or C57BL6/J mice treated with CCl4 (three times a week for 4 weeks; or corn oil for controls), and fed a normal or a PGZ-supplemented diet (0.01% wt/wt). PGZ treatment was associated with increased serum adiponectin concentrations but did not decrease the severity of hepatic fibrosis induced by CCl4. Our data demonstrate that, although having anti-fibrotic properties in rats, PPARγ agonists do not prevent activation of HSCs in vitro, nor hepatic fibrogenesis in vivo in mice.

**Introduction**

Liver fibrosis is the consequence of most types of chronic liver disease and results from excess deposition of extracellular matrix (ECM) components generated mainly by hepatic stellate cells (HSCs) (1,2). In the normal liver, HSCs play a key role in the storage and transport of vitamin A (3). In response to liver injury, or spontaneously when cultured on plastic, these cells activate or trans-differentiate into myofibroblastic-like cells, acquiring contractile, proliferative, pro-inflammatory and fibrogenic properties (4,5). Most of the anti-fibrotic treatments currently under evaluation aim at inhibiting the activation of HSCs (6,7). The identification of key cytokines, transcription factors and molecular mechanisms controlling HSC activation is believed to lead to pathophysiological-based strategies to treat liver fibrosis.

Peroxisome proliferator-activated receptor γ (PPARγ), a member of the steroid/retinoid nuclear hormone receptor superfamily of ligand-activated transcription factors (8), has been proposed as a potential molecular target for inhibition of HSC trans-differentiation. PPARγ has been shown to be expressed in quiescent HSCs and its expression and activity decreased during HSC activation in vitro and in vivo in rats (6,7,9). The treatment of rat HSCs with ligands for PPARγ prevented their activation in vitro. In addition, forced expression of PPARγ in culture-activated HSCs by means of adenoviral transfection has been shown to revert their phenotype to that of quiescent cells (10-12).

In vivo, treatment of rats with thiazolidinediones drugs [Pioglitazone (PGZ) or rosiglitazone], which are synthetic ligands for PPARγ, prevent hepatic fibrosis resulting from chronic toxic injury or bile duct ligation (6,7,13). Despite this potent preventive effect, PGZ has limited efficacy in the treatment of pre-established hepatic fibrosis. Indeed, we recently published that PGZ halts the progression of hepatic fibrosis only when the treatment is administered early in the course of toxic or metabolic disease (14).

PPARγ activation plays a role in various physiological and pathophysiological events, including the stimulation of

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**Abbreviations:** 15d-PGJ1, 15-deoxy-Δ12,14 prostaglandin J1; α-SMA, α-smooth muscle actin; Col-αI, Collagen-αI; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; ECM, extracellular matrix; FBS, foetal bovine serum; HSC, hepatic stellate cell; PGZ, Pioglitazone; PPARγ, peroxisome proliferator-activated receptor γ

**Key words:** PPARγ, hepatic stellate cell, Pioglitazone, hepatic fibrogenesis, mouse
Moreover, although we found PPAR\(\gamma\) in rats, PGZ did not prevent hepatic fibrosis in mice.

PGZ-supplemented diet (CCl\(_4\)+PGZ group). The PGZ-standard diet to obtain a 0.01% Pioglitazone diet (Takeda Chemical Industries, Osaka, Japan) with the powdered supplemented diet was prepared by mixing PGZ (Takeda) with corn oil (ethanol 0.5%). Treatment was initiated at day 1 or day 3 after plating as indicated in the Results section. Medium was renewed every 2 days and the cells were passaged at day 9.

HSC treatment. To analyse the effect of PPAR\(\gamma\) agonists on HSC biology, HSCs were incubated with DMEM supplemented with 5% FBS containing PGZ 10 μM (Takeda Chemical Industries), or vehicle (DMSO). In separate experiments, cells were incubated with the most potent PPAR\(\gamma\) activator 15-deoxy-\(\Delta\)12,14 prostaglandin J\(_1\) (15d-PGl\(_1\)) 10 μM (Sigma Aldrich, ref. no. D8440, Bornem, Belgium) or vehicle (ethanol 0.5%). Treatment was initiated at day 1 or day 3 after plating as indicated in the Results section. Medium was renewed every 2 days and the cells were passaged at day 9.

Materials and methods

Animal studies. Female Balb/C and C57BL6/J mice, were obtained from Elevage Janvier (Le Genest Saint Isle, France). All animals were kept in a temperature- and humidity-controlled environment in a 12-h light-dark cycle. They were allowed free access to water and food. The animals were handled according to the guidelines for humane care for laboratory animals established by the Université Catholique de Louvain (UCL), and the study protocol was approved by the local ethics committee. To examine the effect of Pioglitazone (PGZ) on hepatic fibrosis, 8-week-old Balb/C mice were randomly divided into 4 experimental groups (n=5 per group). Mice were injected with CCl\(_4\) (400 μl/kg body weight in corn oil) intra-peritoneally three times a week for 4 weeks and fed a normal diet (control (17)). The cycle conditions were 94°C for 5 min, 94°C for 1 min, 56°C for 1 min and 72°C for 1 min (30 cycles) and then 72°C for 10 min.

To quantify gene expression, real-time PCR analysis was carried out with the GeneAmp 5700 sequence detection system and software (Applied Biosystems, Den Helder, Netherlands) using SYBR-Green for detection. RPL19 RNA was chosen as an invariant standard. Adiponectin, CD36, Collagen-α1 (Col-α1), PPAR\(\gamma\) total and RPL19 primers were designed using the Primer Express design software (Applied Biosystems). The following primers were used: adiponectin: sense, 5'-gcaagatgagcactctgga-3' and anti-sense, 5'-ccctcgagctctatcctc-3'; CD36: sense, 5'-tcctggaagcatcctcac-3' and anti-sense, 5'-tctggactgaaatgtaactc-3'; Col-α1: sense, 5'-
ttcactacgacagtgt-3' and anti-sense, 5'-ctctgactcaaaaaaacca
ccaaga-3'; PPARγ total: sense, 5'-ctgctcaagatgctttga-3'
and anti-sense, 5'-gttaaagaggtgctctctca-3'; and RPL19:
sense, 5'-gaaatgtaaagaggtttggctc-3' and anti-sense, 5'-
cacaagctgagccagaaag-3'. cDNA derived from adipose tissue
or the liver was used to prepare standard dilution curves.
PCR reactions were performed according to the standardized
thermal profile of the system previously set by the
manufacturer. All tissue and standard curve samples were run
in duplicate at the same time in a single 96-well reaction
plate (MicroAmp Optical, Applied Biosystems) using the
appropriate primers. Results are expressed as relative gene
expression (RGE) using the ΔCt method (User bulletin 2,
Applied Biosystems).

Histology, immunohistochemistry and immunocyto-
chemistry. Mouse livers were fixed in 4% neutral buffered
formalin overnight, processed to paraffin blocks, sectioned,
and stained with hematoxylin and eosin or sirius red (to
visualize collagen deposition) by using standard techniques.
Immunodetection for α-smooth muscle actin (α-SMA) was
performed as previously described (18), using mouse
monoclonal anti-α-SMA (Clone 1A4; 1:100) and the
EnVision Dako mouse as secondary antibody. All reagents
were from DakoCytomation (Via Real, USA), unless
indicated otherwise.

For immunocytochemistry experiments, the cells were
seeded on plastic Thermofax™ coverslips (25 mm in
diameter, Nunc, Rochester, NY, USA). At determined times
(ranging from 1 to 12 days) the coverslips were washed once
in PBS and the cells were fixed by immersion in formaldehyde
4% at 4˚C for 5 min. Endogenous peroxidases were inhibited
by incubating the coverslips in methanol with 0.1% H2O2.
After immersion in PBS containing 10% normal goat serum
for 20 min, the coverslips were incubated with either mouse
monoclonal anti-α-SMA antibody (1:300) (Clone 1A4),
mouse monoclonal anti-Desmin antibody (1:500) (Clone
D33), rabbit polyclonal anti-GFAP antibody (1:1000) (Clone
6F2) or anti-PPARγ antibody (1:50) (H-100; Santa-Cruz,
Heidelberg, Germany) overnight at 4˚C. After washing in PBS,
the coverslips were incubated with the appropriate peroxidase-
conjugated secondary antibody at room temperature for 1 h.
 Peroxidase activity was revealed with 3-amin-9-ethyl-
carbazole (DakoCytomation). Slides were then counter-stained
in haematoxylin and eosin and examined under the microscope.
The percentage of positive cells was determined by counting
the stained cells versus the total number of cells.

Preparation of cell lysates and nuclear extracts. Lysates for
Western blot analyses were obtained by incubating the cells
in 500 μl of buffer containing 150 mM NaCl; 1.5 mM
MgCl2; 10% Glycerol; 0.1% Triton X-100; 1 mM EGTA;
50 mM HEPES and 2 μg/ml of aprotinin and of leupeptin;
for 5 min at 37˚C. The resulting lysates were collected and
clarified by centrifugation (10 min at 12,000 x g). Nuclear
extracts were prepared using the nuclear extract kit (Active
Motif, Rixensart, Belgium), and stored at -80˚C. The protein
contents were determined using a BCA protein assay with
serum albumin as a standard (Pierce Chemical, Rockford, IL.,
USA).

Western blotting. Proteins from HSC lysates were separated
by SDS-PAGE and transferred onto PVDF transfer
membranes (PolyScreen). Fifty-five micrograms of proteins
were loaded for the detection of PPARγ and α-SMA. Homogenates from adipose tissue were used as positive
controls for PPARγ Western blots. The membranes were then
stripped (Pierce’s restore Western blot stripping, Milwaukee,
WI, USA) and reprobed with β-actin antibody to assess equal
protein load. The following antibodies and conditions were
used: a rabbit polyclonal anti-PPARγ antibody (H-100;
Santa-Cruz, Heidelberg, Germany; 1:1000; overnight), a
mouse monoclonal anti-α-SMA antibody (Clone 1A4,
DakoCytomation, 1:5000; 1 h); a mouse monoclonal anti-
β-actin antibody (Clone AC-15, Sigma, Heidelberg, Germany;
1:80000; 1 h); secondary peroxidase-conjugated AffiniPure
goa anti-mouse or goat anti-rabbit antibodies (Jackson
ImmunoResearch, Cambridgeshire, UK) for β-actin and α-SMA
or PPARγ, respectively. The antigen-antibody reaction was
visualized using the Western lighting chemiluminescence
reagent plus (PerkinElmer) detection system followed by
exposure of the membranes to a Kodak X-Omat Blue XB
film. The quantification of immune-reactive proteins was
obtained by densitometry using the Gel Doc 2000 device and
software (Bio-Rad, Nazareth, Belgium).

Electrophoretic mobility shift assays (EMSA). Ten
micrograms of nuclear proteins were incubated for 20 min on
ice in binding buffer (Tris-HCl 10 mM pH 7.5, NaCl 50 mM,
EDTA 1 mM, DTT 1 mM, glycerol 5%) containing 1 μg
poly(dI-dC) (2 μg/ml) and 0.5 ng of γ-32P end-labelled
double-stranded oligonucleotides (20.000 cpm). Nuclear
extracts (5 μg) from COS-7 transfected with PPARγ (Active
Motif, Rixensart, Belgium) were used as positive control.
DNA protein complexes were separated by electrophoresis
(200 V, 2 h) on a 5% polyacrylamide gel (29:1 cross-linking)
in a 1X TBE buffer (88 mmol/l TRIS, 88 mmol/l boric acid,
2 mmol/l EDTA). Gels were dried and exposed to a Kodak
BioMax MS Film (Amersham International plc, Little
Chalfont, UK) for 24 h. Pre-annealed chromatography-
purified double-stranded oligonucleotides containing the
peroxisome-proliferator-responsive element (PPRE) (5'-TGC
ACATTTCAAGAGAGAGAGATTGA-3') were used as a probe (7).
To confirm the identity of the protein/DNA binding,
a supershift analysis was performed as follows: NE
were pre-incubated with 2 μl of specific antibody (2 μg/ml)
(PPARγ; H-100; Santa-Cruz) for 20 min on ice. Then the
binding buffer, dl-dC and probe were added and the mixture
was incubated for a further 20 min on ice.

Biochemical assays. The level of adiponectin in serum was
determined using a mouse adiponectin RIA kit (Linco
Research, MO, USA). The hydroxyproline content was
determined on liver samples hydrolyzed in 5 ml 6 M HCl at
110˚C for 18-24 h as previously reported (19) using hydroxy-
proline 40 μg/ml (Sigma Aldrich) as a standard.

Statistical analysis. Results are expressed as mean ± standard
deviation (SD). Statistical differences between groups were
tested using one way analysis of variance (ANOVA).
Statistical significance was assumed for p values <0.05.
Results

**PPARγ expression in cultured HSCs.** We evaluated the expression of PPARγ in quiescent HSCs (day 3) and its regulation during activation of HSCs under culture conditions. Quiescent HSCs and HSCs cultured for up to 9 days expressed PPARγ mRNA at similar levels (Fig. 1A). After 12 days in culture (i.e. after one passage at day 9), we observed a decreased expression of PPARγ mRNA (Fig. 1A). The decreased expression of PPARγ mRNA after long-term culture and passage has been confirmed by quantification of PPARγ transcripts by real-time PCR (not shown). We then evaluated the expression of PPARγ protein by Western blotting. The presence of PPARγ protein was readily demonstrated in HSCs cultured for up to 5 days, but the protein was no longer detected in HSCs cultured for longer periods (6, 7 or 8 days) (Fig. 1B). As demonstrated by α-SMA expression, the loss of PPARγ protein detection occurred when HSCs underwent activation into myofibroblasts. Thus, after 5 days in culture, there was a marked decrease in PPARγ protein expression, while transcript expression was maintained for up to 9 days, suggesting that the inhibition of protein occurs first at the post-transcriptional then at the transcriptional level during the culture activation of HSCs.

**Functionality of PPARγ in cultured HSCs.** To assess whether the PPARγ transcription factor is functionally active in HSCs, we analysed the mRNA expression of a PPARγ-regulated gene, CD36, in cultured HSCs by real-time PCR. Primary HSCs have been cultured from day 1 to 7 in DMEM supplemented with Pioglitazone (PGZ) 10 μM or DMSO (vehicle), or in the presence of 15d-PGJ2 10 μM or ethanol 0.5% (vehicle). Results have been normalised to the expression of RPL19 mRNA as an invariant control. Data are expressed as mean ± SD on n=10 culture dishes from 2 different cell preparations. p values are given for PPARγ agonist-treated cells versus their respective controls. (B) Electromobility shift assay and supershift on nuclear fractions of 3-day-old HSC exposed for 2 h to PGZ (10 μM) or DMSO as vehicle, or to 15d-PGJ2 or ethanol as vehicle. Nuclear extracts from PPARγ-transfected COS-7 cells were used as positive control. (NS, non specific bands).
retarded band was of stronger intensity in 15d-PGJ₂-treated cells than in the ethanol controls. To note, a PPRE binding complex was seen in ethanol- but not in DMSO-treated cells. Supershift assay confirmed the presence of PPARγ in the protein/DNA retarded complex.

**Effect of PPARγ ligands on PPARγ protein expression in cultured HSCs.** To evaluate the impact of PPARγ ligand on PPARγ protein expression in our culture system, murine primary HSCs were cultured with PGZ (10 μM), or vehicle 24 h after seeding. Lysates were prepared at day 3 and day 7, and used for Western blot analysis. At day 3, PPARγ protein was expressed at similar intensity in HSCs whether cultured in the presence or in the absence of PGZ (Fig. 3). At day 7, PPARγ protein was not detected in control cells. However, culture of HSCs in the presence of PGZ was associated with the persistence of PPARγ protein expression (Fig. 3). This treatment did not affect the trans-differentiation of the cells as confirmed by the α-SMA protein expression in the different groups of treated HSCs (Fig. 3).

**Effects of PPARγ agonists on HSC activation.** To assess the effects of PPARγ activation on fibrogenesis in vitro, we evaluated Collagen-I mRNA expression by real-time PCR in HSCs cultured for 4 days in the presence of PGZ (10 μM) or 15d-PGJ₂ (10 μM). As shown in Fig. 4A and B, treatment with PGZ or 15d-PGJ₂ from day 3-7 did not affect the expression of Collagen-I mRNA, relative to vehicle-treated cells. Similarly, no change in Collagen-I mRNA was seen in HSCs exposed to PPARγ ligands as early as 24 h after seeding (from day 1-5).

As expected, the proportion of HSCs expressing α-SMA increased progressively with time in culture so that 22 and 76% of HSCs were α-SMA positive at day 3 and 7, respectively. To evaluate the impact of PGZ treatment on HSC activation, we incubated the cells with PGZ (10 μM) or DMSO 24 h after plating and evaluated the number of α-SMA expressing cells at day 3 and day 7. As shown in Fig. 4C, the percentage of HSCs expressing the α-SMA protein was similar in the PGZ and the DMSO-treated HSCs, demonstrating that PGZ does not prevent the spontaneous activation of murine HSCs in culture.

**Effect of PPARγ agonist PGZ on hepatic fibrogenesis in vivo.** To determine whether PGZ has anti-fibrotic properties in vivo in mice, we induced hepatic fibrosis in female Balb/C or

Figure 3. Effect of PGZ treatment on PPARγ and α-SMA protein expression in murine HSC. Representative Western blot for PPARγ in HSC cultured from day 1 to day 7 in DMEM containing 5% FBS supplemented with PGZ 10 μM (+) or DMSO as vehicle (-). Cell lysates were prepared at day 3 or at day 7. The initial blot has been stripped and reprobed with α-smooth muscle actin (α-SMA) and β-actin antibodies. Proteins extracted from adipose tissue (FAT) served as positive control.

Figure 4. PPARγ ligands PGZ and 15d-PGJ₂ do not alter Collagen-I mRNA expression or α-SMA expression during culture activation of murine HSC. Graph bars represent collagen mRNA expression quantitated by real-time PCR in primary HSC cultured in the presence of (A) PGZ 10 μM (open bars) or DMSO (vehicle, black bars); or (B) 15d-PGJ₂ (hatched bars) or ethanol 0.5% (vehicle, grey bars). Cells were exposed to PPARγ ligand or vehicle for 4 days (i.e. from day 1-5, or from day 3-7). Individual values represent the ratio of Collagen-I mRNA to RPL19 mRNA. The value obtained for cDNA derived from 2-day-old untreated HSC has been arbitrarily set at one. Data are mean ± SD (arbitrary units) for n=10 dishes per group, from 3 different cell preparations. (C) α-SMA immunopositive cells (% of total cell number) in HSC cultured in the presence of PGZ 10 μM (open bars) or DMSO (vehicle, black bars) for 3 or 7 days. ***p<0.001 compared to 2-day-old, untreated HSC. There is no significant difference between PGZ- and DMSO-treated cells, nor between 15d-PGJ₂- and ethanol-treated HSC.
C57BL6/J mice by repeated intra-peritoneal injections of CCl4 for 4 weeks. During this period, mice were fed a PGZ-supplemented diet (0.01% wt/wt) (n=5) or a standard mouse chow (n=5). Mice fed a PGZ-supplemented diet or a standard chow and injected with corn oil served as non-fibrotic controls. In CCl4-Balb/C mice, treatment with PGZ induced a 3-fold induction of adiponectin mRNA expression in adipose tissue (4.39±2.21 versus 1.19±0.7 arbitrary units in controls; p=0.03). This was paralleled by a 3-fold increase in serum adiponectin in PGZ-treated versus untreated CCl4-Balb/C mice (Fig. 5). Administration of CCl4 to Balb/C mice induced liver fibrosis with formation of collagen bridges between central veins (Fig. 6A). PGZ treatment did not significantly alter the severity of fibrosis induced by CCl4 as demonstrated by Sirius red staining (Fig. 6B). Moreover, the amount and distribution of activated HSCs expressing α-SMA were not different whether CCl4-Balb/C mice were treated or not with PGZ (Fig. 6C and D). In keeping with the histological findings, hepatic hydroxyproline content (Fig. 6E) and induction of hepatic Collagen-I mRNA (Fig. 6F) were at least as high in PGZ-treated than in untreated CCl4-Balb/C mice.

To ensure that this absence of anti-fibrotic effect was not strain-dependent, we repeated the experiment on C57BL6/J mice. As in the Balb/C mice, PGZ treatment of the C57BL6J mice induced the expression of adiponectin transcripts in adipose tissue (not shown) and circulating levels of adiponectin (Fig. 5). As reported by others (20), a similar CCl4 regimen resulted in liver fibrosis of a lesser severity in C57LB6/J than in Balb/C mice demonstrated by peri-centrallobular fibrosis with incomplete bridges (not shown), lower hepatic hydroxyproline content (Fig. 6E) and lower levels of hepatic Collagen-I mRNA (Fig. 6F). Thus, as observed in Balb/C mice, PGZ treatment did not reduce the severity of CCl4-induced fibrosis in C57BL6/J mice.

Discussion

The thiazolidinediones (TZDs), a new class of insulin sensitising anti-hyperglycemic agents, have been identified as high-affinity ligands for PPARγ, a nuclear hormone receptor that plays a central role as a regulator of terminal adipocyte differentiation (21). It has been shown that, in rats, TZDs significantly reduce the severity of hepatic fibrosis (7). This raises the possibility that TZDs may be used for the
prevention and treatment of hepatic fibrosis, and as drugs of particular interest for fibrosis associated with the metabolic syndrome or non-alcoholic steatohepatitis.

Based on their studies of rat HSCs, Tsukamoto and group have proposed the concept that PPARγ is a key regulator of HSC biology, maintaining HSCs in their quiescent phenotype (10). In accordance with observations in rat cells (9), we found PPARγ protein to be expressed in quiescent primary HSCs of mouse origin. Exposure of these cells to PPARγ agonist PGZ activated the transcription factor as demonstrated by the induction of the PPARγ/PPRE binding complex by EMSA as well as by induced expression of CD36, a PPARγ-regulated gene. As in rat cells, cultured-activation of mouse HSCs was associated with a decreased expression of PPARγ, and their treatment with PPARγ ligand prevented the reduction in PPARγ protein expression during culture-induced activation. Despite the presence of functional and ligand-responsive PPARγ, the activation of this transcription factor failed to prevent, or even attenuate, the activation of mouse HSCs in vitro, as it did in rat cells (7,9). Indeed, induction of Collagen-I mRNA and of α-SMA during culture was not modified by exposure of the cells to PPARγ ligands PGZ or 15d-PGJ2.

In rats, two specific mechanisms have been proposed to explain the anti-fibrotic effect of PPARγ agonists. The first one is based on the observations that PPARγ expression is lost in activated HSCs and that forced expression of PPARγ by means of viral transfection is sufficient to revert HSCs to their quiescent phenotype. By analogy to the adipogenic role of PPARγ on adipocytes, this transcription factor might thus be necessary for HSCs to retain their ‘adipogenic’ phenotype, which is characterized by their ability to concentrate vitamin A in lipid droplets and by their quiescence in terms of the proliferation and production of extracellular matrix. The second mechanism involves the direct participation of PPARγ in the transcriptional regulation of genes specific to fibrogenesis. In a recent study, Yavrom et al. showed that, although no PPRE sequence was found in the promoter of the Collagen-I gene of mouse origin, exposure of these cells to PPARγ agonist PGZ activated the transcription factor as demonstrated by the induction of the PPARγ/PPRE binding complex by EMSA as well as by induced expression of CD36, a PPARγ-regulated gene. As in rat cells, cultured-activation of mouse HSCs was associated with a decreased expression of PPARγ, and their treatment with PPARγ ligand prevented the reduction in PPARγ protein expression during culture-induced activation. Despite the presence of functional and ligand-responsive PPARγ, the activation of this transcription factor failed to prevent, or even attenuate, the activation of mouse HSCs in vitro, as it did in rat cells (7,9). Indeed, induction of Collagen-I mRNA and of α-SMA during culture was not modified by exposure of the cells to PPARγ ligands PGZ or 15d-PGJ2.

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We show here, in two different strains of mice, that PPARγ agonist PGZ did not exert anti-fibrotic properties in mice in vivo. Indeed, PGZ had no impact on hepatic fibrosis, induction of hepatic Collagen-I gene expression or activation of hepatic stellate cells induced by chronic administration of CCL4. This is in sharp contrast with several reports on the anti-fibrotic properties of PPARγ agonist drugs in various models of fibrosis in rats (6,7,13). In the literature, this anti-fibrotic effect has been related to inhibition of HSC activation (7,9,13). Therefore, if direct regulation of HSC biology is the principal mechanism implicated in the anti-fibrotic effect of PPARγ agonists in rats, the absence of preventive effect of PGZ on hepatic fibrosis in vivo in mice is not surprising in light of our in vitro results.

A common observation in all studies published to date is that TZD treatment induces the expression of an adipocyte-specific secretory protein, adiponectin (23). Adiponectin has anti-fibrotic properties. Mice lacking adiponectin are exquisitely sensitive to hepatic fibrosis while supraphysiological levels of adiponectin induced by adenovirus infection prevent CCL4-induced fibrosis in wild-type mice (24). In our study, as in similar studies in mice (23,25), PGZ administered as a food mixture increased the expression of adiponectin mRNA in adipose tissue as well as serum levels of adiponectin by a factor 2 to 3. Despite this, PGZ did not protect against fibrosis. This suggests that the rise in adiponectin levels, as obtained by PGZ treatment, is insufficient to provide protection against hepatic fibrosis. Unfortunately, the increase of PGZ doses above the therapeutic range used in this study does not further increase adiponectin levels (25). Alternatively, as PPARγ regulates many functions in adipocytes (24), as well as in inflammatory cells (26,27), the fibro-protective properties of adiponectin may be counterbalanced by the fibro-permissive effect of other factors regulated by PPARγ.

In summary, ligand activation of PPARγ by PGZ does not prevent hepatic fibrogenesis in mice. This is related to the fact that the transcription factor does not control the activation of murine HSCs nor the transcription of fibrogenic genes such as Collagen-I. In addition, the modulation of the production of adiponectin by therapeutic doses of PGZ has a negligible impact on hepatic fibrogenesis.

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