Liver-Specific PPARα-Target Gene Regulation by the Angiotensin Type 1 Receptor Blocker Telmisartan

Markus Clemenz*,1, Nikolaj Frost*,1, Michael Schupp2, Sandrine Caron3, Anna Foryst-Ludwig1, Christian Böhm1, Martin Hartge1, Ronald Gust4, Bart Staels3, Thomas Unger1, Ulrich Kintscher1

* These authors contributed equally to this work

1Center for Cardiovascular Research (CCR), Institute of Pharmacology, Charité-Universitätsmedizin Berlin, Germany
2Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania, USA
3UR 545 INSERM, Institute Pasteur de Lille, Université de Lille 2, Lille, France
4Institute of Pharmacy, Free University of Berlin, Germany

Running Title: Telmisartan and PPARα

Corresponding author:
Prof. Ulrich Kintscher, M.D.
Center for Cardiovascular Research (CCR)
Institute of Pharmacology, Charité – Universitätsmedizin Berlin
Hessische Str. 3-4, 10115 Berlin, Germany
ulrich.kintscher@charite.de

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ABSTRACT

Objective: The angiotensin type 1 receptor blocker (ARB) and PPARγ-modulator telmisartan has been recently demonstrated to reduce plasma triglycerides in non-diabetic and diabetic hypertensive patients. The present study investigates the molecular mechanisms of telmisartan’s hypolipidemic actions, in particular its effect on the PPARα pathway.

Research Design and Methods: Regulation of PPARα-target genes by telmisartan was studied by real-time PCR and Western immunoblotting in-vitro and in-vivo in liver/skeletal muscle of mice with diet-induced obesity (DIO). Activation of the PPARα-ligand binding domain (LBD) was investigated using transactivation assays.

Results: Telmisartan significantly induced the PPARα target genes carnitine palmitoyl transferase 1A (CPT1A) in human HepG2 cells and acyl-CoA synthetase long-chain family member 1 (ACSL1) in murine AML12 cells in the µ-molar range. Telmisartan-induced CPT1A stimulation was markedly reduced after siRNA-mediated knockdown of PPARα. Telmisartan consistently activated the PPARα-LBD as a partial PPARα agonist. Despite high in-vitro concentrations required for PPARα activation, telmisartan (3mg/kg/d) potently increased ACSL1 and CPT1A expression in liver from DIO-mice associated with a marked decrease of hepatic- and serum triglycerides. Muscular CPT1B expression was not affected. Tissue specificity of telmisartan-induced PPARα-target gene induction may be the result of previously reported high hepatic concentrations of telmisartan.

Conclusions: The present study identifies the ARB/PPARγ modulator telmisartan as a partial PPARα agonist. As a result of its particular pharmacokinetic profile, PPARα activation by telmisartan seems to be restricted to the liver. Hepatic PPARα activation may provide an explanation for telmisartan’s anti-dyslipidemic actions observed in recent clinical trials.
Angiotensin type 1 receptor blockers (ARBs) are commonly used in the treatment of hypertension and related cardiovascular end organ damage (1). Recently a distinct subgroup of ARBs has been identified as partial agonists for the peroxisome proliferator-activated receptor γ (PPARγ) with selective PPARγ modulating properties. (2-4) In contrast to full glitazone agonists, PPARγ-activating ARBs exert selective recruitment of nuclear cofactors resulting in in-vivo insulin sensitization in the absence of weight gain in obese insulin resistant mice. (3) Among the ARBs telmisartan has been shown to be the most potent PPARγ modulator. (3; 4) Based on these in-vitro results and data from animal experiments a number of clinical studies have been conducted where the metabolic actions of the PPARγ-activating ARB telmisartan have been intensively investigated. (5-9) When compared to ARBs which do not exert PPARγ-activating properties, telmisartan not only improves insulin sensitivity but also induces beneficial actions on serum lipid levels such as a reduction of serum triglycerides. (5; 10; 11)

PPARs are ligand-activated transcription factors belonging to the superfamily of nuclear receptors. PPARγ is abundantly expressed in adipose tissue and a major regulator of insulin- and glucose metabolism. (12) In contrast, PPARα is highly expressed in tissues displaying a high metabolic rate of fatty acids (FA) such as the liver and skeletal muscle. (13) PPARα modulates intracellular lipid metabolism by transcriptional regulation of genes involved in FA-uptake, mitochondrial FA-oxidation, and triglyceride catabolism. (13; 14) Natural PPARα ligands comprise mono- and polyunsaturated FAs as well as eicosanoids. (15) In addition, PPARα is also the molecular target of the lipid-lowering fibrates such as gemfibrozil, bezafibrate, clofibrate and fenofibrate. These substances are used to treat dyslipidemia and cardiovascular disease. (13; 15)

It has been reported that certain PPARγ activators such as pioglitazone are also able to activate PPARα. Furthermore, it has been proposed that the positive actions of pioglitazone on diabetic dyslipidemia might at least in part be mediated by its PPARα-activating abilities. (16; 17) To understand the underlying mechanism of telmisartan’s lipid-lowering actions we studied the effect of telmisartan on major PPARα-target genes involved in FA-oxidation in the human hepatoma cell line HepG2, the murine hepatic cell line AML12, and in liver/skeletal muscle of diet-induced obese mice treated with telmisartan. Furthermore, activation of the PPARα-ligand binding domain (LBD) and regulation of PPARα protein-/mRNA-expression by telmisartan was studied.

The present study demonstrates that telmisartan induces the PPARα target gene carnitine palmitoyl transferase 1 (CPT1A) in HepG2 cells and acyl-CoA synthetase long-chain family member 1 (ACSL1) in AML12 cells. Consistently, telmisartan acts as a partial PPARα agonist in PPARα transactivation assays and induces PPARα expression. High-fat diet fed mice treated with telmisartan showed a pronounced induction of hepatic ACSL1 and CPT1A which was associated with a significant decrease of hepatic- and serum triglycerides. Interestingly, CPT1B expression in skeletal muscle was not affected by telmisartan. Tissue specificity of telmisartan-induced PPARα-target gene induction may result from high hepatic telmisartan concentrations which have been documented in rodents during early preclinical studies. (18)

In summary, the present study identifies the ARB telmisartan as a partial PPARα agonist. Based on its specific pharmacokinetic profile, PPARα activation by telmisartan appears to be liver-specific. Hepatic induction of PPARα-target genes
involved in mitochondrial FA-oxidation might contribute to the anti-dyslipidemic actions of telmisartan.

**MATERIAL AND METHODS**

**Cell culture.** HepG2, AML12 cells and COS7 cells were cultured following the manufacturer’s guidelines. Cells were serum deprived for 16 hours before stimulation with the vehicle (DMSO) or different effectors.

**Quantitative real-time PCR.** Real-time PCR was performed as previously described using an ABI 7000 and Stratagene 3000 MXP PCR cycler with either the SybrGreen or FAM-TAMRA detection system. (3) Primers and probes are shown in the supplemental data (available at http://diabetes.diabetesjournals.org).

**Transfection and luciferase assay.** Transient transfection and luciferase assays were performed as previously described. (3) Cos7 cells were transfected with the use of Lipofectamine 2000 (Invitrogen) with pGal4-hPPARαDEF (hPPARα ligand-binding domain [LBD] fused to Gal4 DBD) and pGal5-TK-pGL3, and 10 ng pRL-CMV, a renilla luciferase control reporter vector. After 4 hours, transfection medium was replaced by 10% FBS DMEM plus the indicated ARBs, Wy14.643, fenofibric acid or vehicle (dimethyl sulfoxide), and luciferase activity was measured after 24 hours.

**Western immunoblotting.** Electrophoresis and blotting following protein isolation from murine liver tissue was performed as previously described. (3) Blots were incubated with an anti-PPARα (Sigma) or anti-ACSL1 (kindly provided by Rosalind A. Coleman, Chapel Hill, USA) antibody.

**Gene silencing with small interfering RNA (siRNA).** The siRNA targeting human PPARα was purchased from Dharmacon (USA) and two sequences (D-003434-01, D-003434-02) were used simultaneously according to the manufacturer's instructions. The siRNA negative control from Dharmacon (D-001810-01) was used to test non-specific effects on gene expression. Overnight starved HepG2 cells were transfected using HiPerfect (Qiagen, USA) according to the manufacturer's instructions in 24-well plates containing 10^5 cells/well with 5 nM siRNA/well (each sequence 2.5 nM). 30 minutes after start of transfection, cells were treated for 48 h with Telmisartan (50µM; 0.5% serum) before RNA analysis.

**Animals.** Male C57BL/6J mice were treated as previously described. (3) Mice aged 4–5 weeks, were purchased from Harlan Winkelmann (Borchen, Germany). All mice were housed in a temperature controlled (25°C) facility with a 12-h light/dark cycle. Mice were fed with a high-fat diet (60% kcal from fat) for 16 weeks followed by randomization to either a vehicle-treated (0.5% Tween80/ H2O, n=6), a telmisartan-treated (3 mg/kg/day, n=6) group, a pioglitazone (10mg/kg/day, n=6). Telmisartan was provided by Boehringer Ingelheim, and pioglitazone was extracted from tablets. Animals were treated by oral gavage for 10 weeks. Before and after treatment, blood samples were collected from overnight-fasted animals by retroorbital venous puncture under isoflurane anesthesia for analysis of serum triglycerides (enzymatic-colorimetric test, Cypress Diagnostics). After the experiment, animals were killed and organs were dissected. All animal procedures were in accordance with institutional guidelines and were approved.

Triglyceride-content in liver was measured as described previously. (19) Briefly, tissues were homogenized in liquid nitrogen and treated with ice-cold chloroform/methanol/water mixture (2:1:0.8) for 2 min. After centrifugation the aqueous layer was removed and the chloroform layer was decanted. The mixture was incubated at 70°C for chloroform clearance, and the residues were dissolved in isopropanol, and assessed for the triglyceride content using an enzymatic-colorimetric test (Cypress Diagnostics) according to the manufacturer’s instructions. For immunohistochemical studies organs were fixed in 4% formalin,
embedded in paraffin and stained with Haematoxylin/Eosin (H&E).

**Statistical Analysis.** ANOVA followed by multiple comparison testing or *t* test were performed for statistical analysis as appropriate. Statistical significance was designated at *P* < 0.05. Values are expressed as mean±SEM (standard error of the mean) or as indicated.

**RESULTS**

**Telmisartan induces PPARα target gene expression in human and murine hepatocytes.** To evaluate whether telmisartan regulates “classical” PPARα target genes, mRNA expression of CPT1A, the rate limiting enzyme of FA oxidation, in HepG2 cells was examined. Telmisartan induced CPT1A mRNA expression after 48h in a dose-dependent manner starting at 10µM (1.5±0.2-fold vs. vehicle-treated cells, *p*<0.05) and reaching a maximum at 50µM telmisartan (2.84±0.63-fold vs. vehicle-treated cells, *p*<0.001 vs. control) (Figure 1A and 1B). Treatment of HepG2 cells with a classical PPARα-agonist Wy-14,643 for 24h resulted in a 6.6±1.6-fold induction of CPT1A mRNA (100 µM, *p*<0.05 vs. vehicle-treated cells, data not shown).

To prove that telmisartan mediates its actions via PPARα activation, HepG2 cells were transfected with PPARα-specific siRNA. After siRNA treatment of HepG2 cells, PPARα mRNA expression was significantly reduced (Figure 1C, small graph). This siRNA knockdown of PPARα resulted in a significant reduction of telmisartan-induced CPT1A mRNA expression compared to control siRNA indicating a PPARα-dependent mechanism of action (Figure 1C).

To determine whether PPARα-target gene regulation by telmisartan was gene- or species-specific, we next studied the expression of ACSL1, the key player in lipid biosynthesis and FA degradation, in the murine hepatic cell line AML12. ACSL1 mRNA was markedly induced by telmisartan (Figure 1D). In contrast to CPT1A induction in HepG2 cells, maximum ACSL1 mRNA upregulation in AML12 cells was already achieved at 10µM 2.4±0.1-fold after 48h incubation with telmisartan 10 µM (*p*<0.001 vs. vehicle, Figure 1D). Wy-14,643 (10µM) resulted in a 1.46±0.12-fold (*p*<0.01 vs. vehicle), and fenofibrate (100µM) in a 1.55±0.38-fold induction of ACSL1 mRNA (*p*<0.05 vs. vehicle) (Figure 1D). The present data demonstrate that telmisartan induces the PPARα target genes CPT1A in HepG2 cells and ACSL1 in AML12 cells.

**Telmisartan activates the PPARα-LBD and acts like a partial PPARα agonist.** In order to prove whether the induction of hepatic PPARα-target genes by telmisartan resulted from a direct activation of PPARα, we examined its ability to directly activate the PPARα-LBD by using a chimeric Gal4-DBD-hPPARα-LBD fusion protein on a Gal4-dependent luciferase reporter. Telmisartan induced the activation of the PPARα-LBD in a concentration dependent manner, reaching a maximum at 50 µM (Figure 2), with 22.5% of the maximum response induced by the reference PPARα agonist Wy-14,643 identifying telmisartan as a partial PPARα agonist. No activation of the PPARα-LBD was achieved with the ARBs irbesartan or losartan (Figure 2). The computed EC50 values for activation are as follows: telmisartan EC50: 21.8 µM; fenofibric acid EC50: 18.2 µM; and Wy-14,643 EC50: 6.4 µM. Here we identify telmisartan as a partial PPARα agonist inducing activation in the µ-molar range.

**Telmisartan Induces hepatic PPARα target gene expression in diet-induced obese mice.** It has been shown previously that after a single oral administration of telmisartan at a dose of 1 mg/kg in rats, telmisartan prominently concentrates in the liver (tissue distribution of 14C-telmisartan 4h after application: liver: 10673.06 ± 1274.93 ng eq/ml, plasma: 218.85 ± 6.08 ng eq/ml, skeletal muscle: 17.55 ± 1.18ng eq/ml) implicating that the high
concentrations required for PPARα activation and target gene regulation observed in-vitro might be achieved in-vivo. (18) To demonstrate that hepatic PPARα activation by telmisartan occurs in-vivo and translates into metabolic changes, high fat diet-fed obese mice were treated with telmisartan 3mg/kg/d or vehicle for 10 weeks and hepatic/ muscular PPARα target gene expression, hepatic triglyceride accumulation, and serum triglyceride levels were determined.

ACSL1 protein expression in liver tissue was markedly increased in telmisartan-treated mice compared to the vehicle-treated animals (Figure 3A). Accordingly, relative ACSL1 mRNA expression increased 2.5±0.3-fold in livers of telmisartan-treated animals (p<0.01 vs. vehicle, Figure 3B). In consonance, telmisartan treatment led to a 3.2±0.4-fold increase of hepatic CPT1A mRNA compared to vehicle-treated mice (p<0.01 vs. vehicle, Figure 3B). Interestingly, telmisartan had no effect on PPARα target gene expression in skeletal muscle (Figure 3C). Together, these results indicate that telmisartan markedly induces hepatic PPARα-target genes involved in FA catabolism in obese mice.

Previously, telmisartan has been characterized as a partial PPARγ agonist. (4) To evaluate the role of hepatic PPARγ activation in the actions of telmisartan, mRNA expression of the PPARγ target gene CD36 and PPARγ2 was analyzed in liver tissue from telmisartan-treated mice (Figure 3D). Hepatic CD36 and PPARγ2 mRNA level were not significantly regulated by telmisartan treatment suggesting the absence of major hepatic PPARγ activation by telmisartan (Figure 3D). Furthermore, we studied the effect of the full PPARγ agonist pioglitazone in livers from HFD-fed mice. Mice treated with pioglitazone (10mg/kg/d) for 10 weeks exhibited no regulation of hepatic CPT1A mRNA expression, whereas the PPARγ target gene CD36 was significantly induced by 2.8±0.3-fold compared to vehicle-treated HFD-fed mice (p<0.01 vs. vehicle-treated HFD-fed mice) (Figure 3E).

**Telmisartan reduces hepatic and serum triglycerides in diet-induced obese mice.** Increased expression of hepatic CPT1A and ACSL1, and subsequent higher rates of hepatic FA oxidation in telmisartan-treated animals should result in decreased accumulation of triglycerides in liver and serum.

Telmisartan treatment prominently reduced liver triglyceride content in obese mice (29.7±11.7µmol/g wet weight) when compared to vehicle-treated mice (79.5±17.4µmol/g wet weight, p<0.005, Figure 4A and 4B). In accordance, serum triglycerides in high fat diet-fed mice declined from 122.3±28.4mg/dL before treatment to 53.9±4.5mg/dL after telmisartan treatment (p<0.005, Figure 4C) implicating that hepatic PPARα gene induction by telmisartan translates into a lowering of systemic and local triglyceride level.

In humans, AST/ ALT ratios are indicative for the extent and etiology of liver damage. (20) Whereas mild liver disease like non-alcoholic fatty liver disease and uncomplicated virus hepatitis are affiliated with ratios <1, severe liver disease like chronic hepatitis, liver cirrhosis and alcoholic fatty liver disease result in increased ratios >1. (20) Firstly, we compared AST/ ALT ratios in mice on a low fat diet (10% kcal from fat) with high fat-diet fed animals. The mean AST/ ALT ratio in low fat diet-fed control mice was 1.1±0.1 which was significantly decreased by high fat diet to 0.5±0.1 (p<0.05). In line with the reduction of liver steatosis, telmisartan treatment restored impaired AST/ ALT ratio to normal levels(1.1±0.3) indicating that reduction in hepatic triglycerides by telmisartan also improved high-fat diet mediated deterioration of liver function.

**Telmisartan induces PPARα expression in vivo and in vitro.** To explore additional mechanisms of PPARα-activation by telmisartan which might be additive to
LBD-dependent activation, we investigated the regulation of PPARα by telmisartan. Liver PPARα protein expression markedly increased with telmisartan treatment (Figure 5A). In accordance, hepatic PPARα mRNA was upregulated 1.9±0.2-fold in the telmisartan-treated animals compared to vehicle-treated mice (p<0.01) (Figure 5B). Moreover, PPARα mRNA induction by telmisartan was observed in HepG2 cells with a maximum induction of 3.4±0.4-fold (p<0.05 vs. vehicle) after 48h at high concentrations of telmisartan (50µM) (Figure 5C). The ARB eprosartan had no effect on PPARα mRNA expression (Figure 5C). These data show that in addition to LBD activation telmisartan is capable of inducing PPARα expression which might contribute to the observed target gene regulation.

DISCUSSION

The present data demonstrate that the ARB telmisartan induces PPARα target genes in human and murine hepatic cells, and acts as a partial PPARα agonist in the higher µ-molar range in-vitro. As a result of its particular pharmacokinetic profile with high concentrations in liver, telmisartan potently induces hepatic PPARα-target genes involved in FA catabolism in obese mice which was associated with a significant reduction of systemic and local triglyceride level.

Derosa and colleagues could demonstrate that telmisartan when compared to eprosartan significantly reduced serum triglycerides in hypertensive type 2 diabetic patients by 24.8% compared to baseline, an effect which may not be fully explained by its AT1-blocking/ PPARγ-modulating actions. (5) Additional studies in diabetic and non-diabetic hypertensive patients have confirmed significant lower plasma triglyceride levels after telmisartan treatment. (10; 11) In the present study we identify telmisartan as a weak PPARα agonist in-vitro. Furthermore, telmisartan treatment in-vivo induced PPARα-regulated genes involved in hepatic FA-oxidation at relatively low doses suggesting that hepatic PPARα activation by telmisartan might be clinically relevant. It is well known that treatment of hypertriglyceridemic patients with fibrates results in potent lowering of triglyceride levels which is, at least in part, mediated via hepatic PPARα activation, subsequent induction of FA-oxidation in the liver, decreased VLDL particle production and plasma triglycerides. (21) In consonance with this, telmisartan also significantly reduced circulating triglyceride levels in our animal model which is also consistent with recent data observed in the leptin receptor-deficient Zucker rat treated with telmisartan. (22) Together, these results underscore that PPARα activation in the liver may contribute to telmisartans action on dyslipidemia in patients.

Telmisartan has been recently characterized as a selective PPARγ modulator. (3; 4) Hepatic activation of PPARγ contributes to the actions of PPARγ agonists on lipid- and glucose metabolism in mice. (23-25) To characterize the relevance of hepatic PPARγ activation for telmisartan’s effects, CD36 and PPARγ2 expression were investigated, and compared to the full PPARγ agonist pioglitazone. Telmisartan did not regulate CD36 or PPARγ2 in livers from HFD-fed mice excluding a major role of hepatic PPARγ in telmisartan’s action. In contrast, pioglitazone failed to induce CPT1A but stimulated CD36 mRNA expression in liver. These data are consistent with previous findings of distinct gene expression profiles in adipocytes treated with telmisartan or piogliatzone. (3) In liver, telmisartan may mainly act as a partial PPARα agonist whereas piogliatzone also activates PPARγ pathways.

Telmisartan reduced hepatic triglyceride content in high-fat diet fed mice. Non-alcoholic steatohepatitis (NASH) frequently develops during obesity as a result of insulin resistance and subsequent hepatic triglyceride overload. (26) NASH is
considered as a hepatic component of the metabolic syndrome leading to liver fibrosis and cirrhosis. (26) Currently no drug therapy has been established for the treatment of NASH. Recently, a number of small clinical trials have demonstrated that the PPARγ agonists rosiglitazone and pioglitazone improve liver histology and aminotransferase levels in patients with NASH. (27-29) PPARα activation by fibrates has been recently reported to reduce the development of NASH in different animal models. (30; 31) Therefore, combination of hepatic PPARα activation and systemic PPARγ modulation by telmisartan, together with the observed reduction of liver triglyceride content, may provide a new therapeutic option for the future treatment of NASH. Telmisartan’s action on hepatic pathology has been already described by Sugimoto and colleagues. (32) In rats fed a high fat, high carbohydrate diet, telmisartan but not valsartan significantly reduced hepatic triglyceride. (32) Along this line, Fujita and colleagues could show that telmisartan application to a rat model of NASH improved numerous pathological features of the disease including liver steatosis, liver inflammation, and liver fibrosis underlining the potential role of telmisartan for NASH therapy. (33) The hepatic actions of telmisartan will gain clinical importance in the light of previous data demonstrating an increased prevalence of fatty liver disease in hypertensive patients. (34) The antihypertensive actions combined with hepato-protective actions of telmisartan could be of added clinical value in these patients.

The pharmacokinetic profile of telmisartan seems to be highly important for its PPARα-activating properties. In preclinical studies with telmisartan, Shimasaki and colleagues have shown that telmisartan prominently concentrates in the liver with approximately 40 times higher levels compared to plasma and skeletal muscle which opens the possibility that the high concentrations required for PPARα activation and target gene regulation observed in-vitro might be achieved in-vivo in a tissue-specific manner. (18) The high liver content of telmisartan is likely caused by binding of the compound to the glutathione-S-transferase (GST) type 1-1 (ligandin) protein which is present at high concentrations in the liver. (35) Hepatic storage may be further supported by the lipophilic characteristic of telmisartan. Compared to the liver, telmisartan concentrations in skeletal muscle are extremely low 4h after administration which makes it unlikely that concentrations required for muscular PPARα activation are reached. (18) In accordance with the pharmacokinetic data, telmisartan did not affect PPARα target gene expression in skeletal muscle of obese mice strongly supporting a tissue-specificity for telmisartan-induced PPARα activity.

The liver-specificity of telmisartan-mediated PPARα activation does not only provide a mechanism for its beneficial effects on triglyceride levels but also plays a major role for potential PPARα-mediated side effects. One of the most common toxic side effect during fibrate therapy are myopathies associated with myalgias, in particular in combination with statin therapy. (36) Since muscular PPARα-target genes are not activated by telmisartan, and telmisartan concentrations in skeletal muscle are minor, the occurrence of PPARα-mediated muscular side effects under telmisartan therapy are very unlikely. Moreover, the unique pharmacokinetic profile of telmisartan allows beneficial liver-specific PPARα activation in the absence of common PPARα-mediated side effects.

In addition to activation of the PPARα-LBD, telmisartan-mediated induction of protein and mRNA expression of PPARα could be detected in-vitro and in-vivo. This data are in accordance with previously published studies, demonstrating a stabilization of the PPARα protein after ligand binding. (37) Here, we identify that
also PPARα mRNA expression is positively regulated by telmisartan suggesting an additional transcriptional mechanism of ligand (telmisartan)-mediated receptor regulation. This is in line with previous reports demonstrating an induction of hepatic PPARα mRNA expression by fibrate treatment in rodents and human hepatocytes. (38-40) Nevertheless, future studies are required to investigate whether PPARα mRNA regulation by telmisartan depends on telmisartan-PPARα LBD interactions or whether this might be mediated via blockade of the AT1 receptor. As yet, it seems that telmisartan positively regulates the PPARα pathway by two different mechanisms: (1) LBD-activation and (2) receptor upregulation.

In summary, the present study identifies the ARB/PPARγ modulator telmisartan as a partial PPARα agonist. As a result of its particular pharmacokinetic profile with high concentration in liver, PPARα activation by telmisartan seems to be restricted to the liver. Hepatic PPARα activation by telmisartan may provide an explanation for its anti-dyslipidemic actions observed in clinical trials and prevents simultaneously potential danger from systemic PPARα-mediated adverse effects. The multimodal mechanism of action of telmisartan including AT1-receptor blockade/ PPARγ modulation and hepatic PPARα activation characterizes this compound as a therapeutic option for the treatment of patients suffering from multiple cardiometabolic disorders such as hypertension, glucose intolerance, and dyslipidemia.

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REFERENCES


FIGURE LEGENDS

Figure 1. Telmisartan induces PPARα target gene expression in human and murine hepatocytes.

A. HepG2 cells were serum deprived for 16h and incubated with telmisartan 50µM. mRNA expression of carnitine palmitoyl transferase 1 (CPT1A) was determined by real-time PCR after the indicated time points. ***p<0.001 vs. vehicle control. B. mRNA expression of CPT1A in HepG2 cells with incubation of telmisartan (0.1, 1, 10 and 50µM) for 48 hours. *p<0.05, ***p<0.001 vs. vehicle control. C. HepG2 cells were serum deprived for 16h and transfected with PPARα siRNA or control siRNA followed by incubation with telmisartan 50µM for 48h. mRNA expression of CPT1A was determined by real-time PCR. *p<0.05 and ***p<0.001 vs. vehicle control with control siRNA, ## p<0.01 vs. telmisartan-treated cells with control siRNA. (small graph: PPARα mRNA expression in HepG2 cells with control/PPARα siRNA, ***p<0.001 vs. control siRNA; data are shown as % of PPARα mRNA expression in control siRNA-transfected cells). D. Murine AML12 cells were incubated with telmisartan (10µM), Wy-14,643 (10 µM) or fenofibric acid (100µM). After 48 hours of incubation acyl-CoA synthetase long-chain family member 1 (ACSL1) mRNA was determined. *** p<0.001, ** p<0.01, * p<0.05 vs. vehicle control. Expression was normalized to 18S expression. Experiments were repeated 4 times and results are presented as x-fold induction over vehicle-treated cells. Mean ± SEM is shown.

Figure 2. Telmisartan activates the PPARα-LBD and acts like a partial PPARα agonist

COS7 cells were transiently transfected with the pGal4-h PPARα -LBD and pGal5- Tk-pGL3 reporter followed by stimulation with the ARBs, fenofibric acid (Feno acid) and Wy-14,643 (Wy14643) as indicated. Firefly luciferase activity was measured after 24 hours and normalized with activity of cotransfected renilla luciferase. Experiments were repeated 3 times; results are presented as mean±SD.

Figure 3. Telmisartan Induces hepatic PPARα target gene expression in diet-induced obese mice

C57BL/6J mice were fed a high-fat diet (HFD, 60% kcal from fat) for 16 weeks followed by 10 wks. treatment with vehicle, telmisartan (3 mg/kg/day), or pioglitazone (10mg/kg/d). At the end of the treatment period, animals were dissected and organs were shock frozen in liquid nitrogen. A. Hepatic ACSL1 protein expression. A representative western immunoblot is shown. B. Relative hepatic mRNA expression of ACSL1 and CPT1A in telmisartan treated animals. **p<0.01 vs. HFD vehicle. C. mRNA expression of CPT1B in skeletal muscle tissue of telmisartan treated animals. n.s. = statistically not significant vs. HFD vehicle animals. D. Relative hepatic mRNA expression of CD36 and PPARγ2 in telmisartan treated animals. n.s. = statistically not significant vs. HFD vehicle animals. E. Relative hepatic mRNA expression of CPT1A and CD36 in pioglitazone-treated animals. n.s. = statistically not significant vs. HFD vehicle animals. **p<0.01 vs. HFD vehicle.

Expression was normalized to 18S expression. Experiments were repeated 4 times and results are presented as x-fold induction over vehicle-treated cells. Mean ± SEM is shown.

Figure 4. Telmisartan reduces hepatic and serum triglycerides in diet-induced obese mice

A. Liver triglyceride content of telmisartan treated and vehicle treated animals on high fat diet (HFD) in µmol/G wet weight. *** p<0.005. B. HE staining of representative liver
sections from vehicle and telmisartan HFD-fed mice. C. Serum triglycerides in HFD-fed mice before and after telmisartan treatment. ***p<0.005 pre vs. post treatment.

Figure 5. Telmisartan induces PPARα expression in vivo and in vitro.

A. PPARα protein expression from liver protein extracts of HFD fed mice treated with telmisartan or vehicle control. A representative western immunoblot is shown. B. PPARα mRNA expression in livers from telmisartan-treated and vehicle-treated HFD animals. **p<0.01 vs. HFD vehicle-treated control. C. PPARα mRNA induction by telmisartan in HepG2 cells. Cells were incubated for 48 hours with telmisartan 1, 10 and 50µM, eprosartan 100µM or vehicle. *p<0.05 vs. vehicle control. Results are presented as x-fold induction over vehicle-treated mice/ cells. Mean ± SEM is shown.
Figure 1

A. CPT1A

B. CPT1A

C. CPT1A

D. ACSL1
Figure 3

A  hepatic ACSL1 protein expression

68 kD

HFD vehicle  Telmisartan 3mg/kg/d
(n=3 mice)

B  hepatic mRNA expression

CPT1A  ACSL1

**

Relative x-fold mRNA expression

HFD vehicle  Telmisartan 3 mg/kg/d

C  CPT1B muscular mRNA expression

Relative x-fold mRNA expression

HFD vehicle  Telmisartan 3 mg/kg/d

D  hepatic mRNA expression

CD36  PPARγ2

n.s.

Relative x-fold mRNA expression

HFD vehicle  Telmisartan 3 mg/kg/d

E  hepatic mRNA expression

CPT1A  CD36

**

Relative x-fold mRNA expression

HFD vehicle  Pioglitazone 10 mg/kg/d

n.s.
Figure 4

A. Liver triglyceride content

<table>
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<tr>
<th>Treatment</th>
<th>Value (μmol/g wet weight)</th>
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<td>HFD vehicle</td>
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</tr>
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<td>Telmisartan 3 mg/kg/d</td>
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B. Liver histology

HFD vehicle

Telmisartan 3 mg/kg/d

C. Serum triglycerides

<table>
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<th>Treatment</th>
<th>Value (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFD pre-treatment</td>
<td>175</td>
</tr>
<tr>
<td>HFD post-treatment</td>
<td>125</td>
</tr>
</tbody>
</table>
Figure 5

A  hepatic PPARα protein expression

B  hepatic PPARα mRNA expression

C  PPARα expression in HepG2 cells

Telmisartan and PPARα