Published in final edited form as:

J Atheroscler Thromb. 2015 August 26; 22(8): 754–772. doi:10.5551/jat.28720.

Transcriptome Analysis of K-877 (a Novel Selective PPAR α Modulator (SPPARM α))-Regulated Genes in Primary Human Hepatocytes and the Mouse Liver

Sana Raza-Iqbal¹, Toshiya Tanaka^{1,2}, Motonobu Anai¹, Takeshi Inagaki^{2,3}, Yoshihiro Matsumura³, Kaori Ikeda², Akashi Taguchi¹, Frank J. Gonzalez⁴, Juro Sakai^{2,3}, Tatsuhiko Kodama¹

¹Laboratory for Systems Biology and Medicine (LSBM), Research Center for Advanced Science and Technology (RCAST), University of Tokyo, Tokyo, Japan

²Translational Systems Biology and Medicine Initiative Center for Disease Biology and Integrative Medicine, Faculty of Medicine, University Tokyo, Tokyo, Japan

³Division of Metabolic Medicine, Research Center for Advanced Science and Technology (RCAST), University of Tokyo, Tokyo, Japan

⁴Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States

Abstract

Aim: Selective PPAR α modulators (SPPARM α) are under development for use as next-generation lipid lowering drugs. In the current study, to predict the pharmacological and toxicological effects of a novel SPPARM α K-877, comprehensive transcriptome analyses of K-877-treated primary human hepatocytes and mouse liver tissue were carried out.

Methods: Total RNA was extracted from the K-877 treated primary human hepatocytes and mouse liver and adopted to the transcriptome analysis. Using a cluster analysis, commonly and species specifically regulated genes were identified. Also, the profile of genes regulated by K-877 and fenofibrate were compared to examine the influence of different SPPARM α on the liver gene expression.

Results: Consequently, a cell-based transactivation assay showed that K-877 activates PPAR α with much greater potency and selectivity than fenofibric acid, the active metabolite of clinically used fenofibrate. K-877 upregulates the expression of several fatty acid β -oxidative genes in human hepatocytes and the mouse liver. Almost all genes up- or downregulated by K-877 treatment in the mouse liver were also regulated by fenofibrate treatment. In contrast, the K-877-regulated genes in the mouse liver were not affected by K-877 treatment in the *Ppara*-null mouse

Address for correspondence: Tatsuhiko Kodama, Laboratory for Systems Biology and Medicine (LSBM), Research Center for Advanced Science and Technology (RCAST), The University of Tokyo, Komaba 4-6-1, Meguro-ku, Tokyo 153-8904, Japan, kodama@lsbm.org.

Conflicts of Interest

Tatsuhiko Kodama is an advisory board member at Kowa Co.

Tatsuhiko Kodama is a recipient of a collaborative research fund from Kowa Co.

liver. Depending on the species, the peroxisomal biogenesis-related gene expression was robustly induced in the K-877-treated mouse liver, but not human hepatocytes, thus suggesting that the clinical dose of K-877 may not induce peroxisome proliferation or liver toxicity in humans. Notably, K-877 significantly induces the expression of clinically beneficial target genes (*VLDLR*, *FGF21*, *ABCA1*, *MBL2*, *ENPEP*) in human hepatocytes.

Conclusion: These results indicate that changes in the gene expression induced by K-877 treatment are mainly mediated through PPAR α activation. K-877 regulates the hepatic gene expression as a SPPARM α and thus may improve dyslipidemia as well as metabolic disorders, such as metabolic syndrome and type 2 diabetes, without untoward side effects.

Keywords

Transcriptome; SPPARM α ; Species difference

Introduction

Continuous disruption of the energy balance due to excess caloric intake and/or reduced energy consumption causes obesity and low-grade inflammation, ultimately leading to the onset of metabolic disorders, such as metabolic syndrome, type 2 diabetes and atherosclerosis¹⁾. Therefore, enhancing lipid catabolism and excretion or reducing lipid absorption and *de novo* synthesis are well established strategies for treating metabolic disorders. These therapies include HMG-CoA reductase inhibitors (statins), bile acid binding resins and fibrates. Fibrates have been demonstrated through a long history of clinical use to ameliorate lipid abnormalities by decreasing the plasma triglyceride (TG) level and increasing the HDL-cholesterol level. However, clinically available fibrates sometimes evoke untoward effects, such as elevation of the transaminase, homocysteine and creatinine levels. Therefore, most fibrates are contraindicated in patients with hepatic or severe renal dysfunction. In addition, limited evidence has been reported with respect to the ability of these agents to prevent macrovascular events in clinical trials²⁾. To generate new chemical entities that maximize the beneficial effects and minimize the adverse effects of fibrates, selective PPAR α modulators (SPPARM α) have been actively pursued as the next generation of lipid-lowering drugs^{3, 4)}.

K-877 is a novel SPPARM α that enhances the PPAR α activity and selectivity by introducing a 2-aminobenzoxazole ring and phenoxyalkyl chain into fibric acid⁵⁾. K-877 has greater PPAR α activation potency than other fibrates, with a lower EC₅₀ value and higher degree of subtype selectivity (>1,000-fold subtype selectivity). In preclinical studies, K-877 has been shown to have a greater TG-lowering effect than fenofibrate in normolipidemic rats with a reduced amount of liver weight gain⁵⁾. Additionally, K-877 treatment induces a more pronounced increase in the plasma h-apoAI levels in human apoAI-I transgenic mice than fenofibrate. Furthermore, K-877 treatment results in a decrease in the atherosclerotic lesion area in *Ldlr*-null mice⁴⁾. Currently K-877 is undergoing a phase III trial in Japan for the treatment of dyslipidemia. Phase II/III studies showed that 0.1 to 0.4 mg/day of K-877 significantly reduced the plasma TG levels (−46.3% to −51.8%) and increased the HDL-cholesterol levels (20.3% to 24.7%). Furthermore, the incidence of adverse events (AEs) and adverse drug reactions (ADRs) in the K-877 treatment group was comparable to that

observed in the placebo and 100 mg/day fenofibrate groups, whereas the incidence of AEs and ADRs in the K-877 treatment group was lower than that in the 200 mg/day fenofibrate treatment group⁶). Therefore, K-877 may replace fibrates as the first clinically available SPPARM α to improve dyslipidemia and prevent macro- and microvascular risks. However, the exact mechanisms underlying the pharmacological and toxicological effects of K-877 are not fully understood.

Aim

In this study, to predict the mode of action, beneficial effects, and untoward effects of K-877, microarray analyses were carried out on K-877 treated primary human hepatocytes and mouse liver.

Materials and Methods

Chemical Reagents

K-877 was kindly provided by Kowa Co. Ltd. Selective high-affinity agonists of PPAR β/δ GW501516⁷), fenofibrate and fenofibric acid were synthesized as previously described⁸). Rosiglitazone (BRL 49653) was purchased from Cayman Chemical. Arabic gum was purchased from Wako Pure Chemical Industries.

Cell Culture

The human hepatoma cell line HepG2 was purchased from ATCC and maintained in DMEM medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS) containing 100 U/mL of penicillin and 100 μ g/mL of streptomycin (Gibco) at 37°C in 5% CO₂. Chinese hamster ovary (CHO) cells were maintained in Ham's F-12 Nutrient Mixture medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS) containing 100 U/mL of penicillin and 100 μ g/mL of streptomycin (Gibco) at 37°C in 5% CO₂.

Primary Human Hepatocytes

Cryopreserved primary human hepatocytes were purchased from Bioreclamation IVT. The clinical characteristics of the donors are shown in Table 1. The hepatocytes were cultured in *In Vitro*GRO CP medium (Bioreclamation IVT) supplemented with Torpedo Antibiotic (Bioreclamation IVT). The cells were diluted to 0.70 $\times$ 10⁶ viable cells/mL, and 2.5 mL/well was cultured in a collagen I-coated 6-well plate. The hepatocyte cultures were maintained at 37°C, 95% humidity and 5% CO₂ for 3 hours and gently replaced with *In Vitro*GRO CP with antibiotics. After 24 hours, the hepatocytes were treated with *In Vitro*GRO CP with antibiotics containing 100 nM and 10 μ M of K-877, 100 μ M of fenofibric acid or 0.01% DMSO as a control.

Construction of the pBIND Vectors

pBIND-hPPAR α -LBD (166–468 aa), pBIND-hPPAR β/δ -LBD (138–441 aa) and pBIND-hPPAR γ 1-LBD (175–478 aa) vectors are high-copy plasmids in which the CMV immediate early promoter drives the expression of the chimeric protein of the yeast Gal4 DNA-binding

domain fused to the PPAR ligand-binding domain. The *Renilla* luciferase gene on these vectors is preceded by the SV40 early promoter. To construct these plasmids, the corresponding regions were amplified via PCR and cloned in-frame within the pBIND vector (Promega).

Luciferase Reporter Assay

CHO and HepG2 cells were transfected with the indicated pBIND vector together with the pG5*luc* reporter plasmid (Promega) which contains five GAL4 binding sites upstream of a minimal TATA box using TransIT LT-1 transfection reagent (Takara). At 24 hours after transfection, the cells were harvested and plated in a 96-well tissue culture plate. The cells were subsequently treated with each compound and, after 24 hours of treatment, were lysed in lysis buffer (Promega) and analyzed using the Dual-Luciferase® Reporter Assay System (Promega). The Firefly luciferase signal was normalized to the Renilla luciferase signal.

Animal Experiments

C57Bl/6Jcl (C57Bl/6J) mice were purchased from CLEA Japan. PPAR α null (*Ppara*-null) mice⁹ were purchased from Jackson laboratories. All animals were housed in a temperature-controlled (24°C) facility with a 12-hour light/dark cycle (08:00 to 20:00 light) and allowed free access to water and standard chow (CE-2; CLEA Japan). K-877 and fenofibrate were orally administered to 7-week-old mice at 10 mL/kg body weight in 3% arabic gum daily between 09:30 and 10:00 for three days. All mice were killed four hours after the final administration.

Transcriptome Microarray Analysis

For the genome-wide transcription analysis, the GeneChip Human Genome U133 Plus 2.0 array and GeneChip Mouse Genome 430 2.0 array were used as previously described^{8, 10}. Briefly, total RNA was extracted with ISOGEN (Nippon Gene Inc.) from the primary human hepatocytes treated with DMSO, K-877 or fenofibric acid and the mice livers treated with vehicle, K-877 or fenofibrate. Following *in vitro* transcription (IVT) and cRNA fragmentation, the fragmented IVT product was hybridized on an array and stained with streptavidin phycoerythrin according to the manufacturer's recommended protocol. The arrays were scanned using the Affymetrix GeneChip Scanner 3000 (Affymetrix), and the GeneChip Analysis Suite software program version 5.0 was used to calculate the average difference for each gene probe.

Quantitative Analysis

The expression level of each gene and the fold change between the experiments were calculated using the GeneChip Analysis Suite software program version 5.0 (Affymetrix). The average difference in each experiment was normalized to 100. The criterion for the significant induction of a particular gene was an average difference equal to or more than 100 for the drug-treated samples and a fold change equal to or greater than 2^{0.6}. The criterion for a significant reduction in a particular gene was an average difference equal to or more than 100 for the vehicle-treated mice liver samples and a fold change equal to or less than 2^{-0.6}.

Statistical Analysis

The homogeneity in variance was evaluated according to Bartlett's test followed by the parametric or non-parametric Dunnett's or Bonferroni's multiple comparison test. * $p < 0.05$, ** $p < 0.01$.

Results

K-877 Selectively Activates PPAR α

To evaluate the therapeutic potential and subtype selectivity of K-877 for activating PPAR α , CHO and HepG2 cells were transiently transfected with Gal4 DBD and the hPPARs-LBD fusion protein expression plasmid, together with the reporter plasmid pG5 luc , which expresses the firefly luciferase gene under the control of five copies of the Gal4 binding site. In the CHO cell-based transactivation assay, K-877 activated human PPAR α in a dose-dependent manner, with an EC₅₀ of 1.5 nM (Fig.1a). K-877 was >2,000-fold selective for PPAR α over the other subtypes (PPAR δ and PPAR γ) in a CHO cell-based transactivation assay. On the other hand, the concentration-activity curves for each PPARs transactivation assay of K-877 were shifted to the right and the maximal activation was lower in the HepG2 cell-based transactivation assay (Fig.1b). These results suggest that the expression levels of PPAR fusion proteins and the reporter gene affect the sensitivity of the cell-based transactivation assay. Hence, to fully activate PPAR α in primary human hepatocytes, we decided to use 100 nM (to selectively fully activate PPAR α in the CHO cell-based assay) and 10 μ M (to fully activate PPAR α in HepG2 cells and barely influence the other subtypes) of K-877 in the further studies.

K-877 Regulates Fatty Acid Metabolic Genes in Primary Human Hepatocytes

To characterize the regulation of the PPAR α target gene expression during K-877 treatment, an oligonucleotide microarray analysis was performed on K-877-treated primary human hepatocytes. The characteristics of the primary human hepatocytes and their response to K-877 and fenofibric acid treatment are shown in Table 1. Sixty-four probe sets representing 50 genes were commonly upregulated by 10 μ M of K-877 treatment. Due to the lower responsiveness of JGM hepatocytes and the stringent criteria used for the analysis, commonly downregulated genes could not be identified. As shown in Table 2, 11 of the top 20 genes upregulated by K-877 treatment were involved in carbohydrate and lipid metabolism. Importantly, these genes were also induced by fenofibric acid treatment. In addition, nine of these 11 genes (except for *AADAC* and *SLC25A42*) have been reported to be direct PPAR α target genes^{11–16}. Arylacetamide deacetylase (*AADAC*) and *SLC25A42* are involved in VLDL assembly¹⁷ and coenzyme A transport¹⁸, respectively, and are important for determining the fatty acid fate. These results indicate that K-877 induces fatty acid catabolism in human hepatocytes.

K-877 Regulates the Gene Expression in a PPAR α -Dependent Manner

To identify genes regulated by K-877 treatment in the mouse liver, the mice were administered 1, 3 and 10 mg/kg/day of K-877 and 100 mg/kg/day of fenofibrate for three days. As revealed by the microarray analysis, K-877 treatment resulted in an increase in the

number of up- or downregulated mRNAs in a dose-dependent manner (Supplementary Table 1). In previous preclinical animal studies^{4, 5}, the effects of 1 to 10 mg/kg/day doses of K-877 were compared with that of 100 mg/kg/day of fenofibrate; 645 and 603 probe sets were upregulated by 3 mg/kg/day of K-877 and 100 mg/kg/day of fenofibrate treatment, respectively, while, 627 and 538 probe sets were downregulated, respectively (Supplementary Table 1). Among these probe sets, 512 and 426 were commonly up- or downregulated (Supplementary Fig.1). Therefore, these results suggest that 3 mg/kg/day of K-877 and 100 mg/kg/day of fenofibrate are almost equivalent in terms of *in vivo* potency with respect to the regulation of the gene expression. These results suggest that mode of action of K-877 and fenofibrate is biologically equivalent.

Table 3 and Table 4 show the top 20 genes that were up- or downregulated in the K-877-treated mouse liver, respectively. Among these genes, 12 genes upregulated by K-877 treatment are involved in carbohydrate and lipid metabolism (Table 3), of which *Cyp4a3l*¹⁹, *Pdk4*¹², *Cidec*²⁰, *Acot1*²¹, *Acot2*²², *Acot3*²³, *Slc27a1* (FATP1)¹³, *Vnn1*^{24, 25} and *Aqp3*²⁶ have been reported to be PPARs target genes. On the other hand, K-877 treatment reduced the expression of phase II enzymes and xenobiotic transporters, such as the *Slco1a4*, *Sult1a1*, *Slc22a7* and *Cyp2c54* genes²⁷ (Table 4).

We next asked whether PPAR α mediates the regulation of the gene expression by K-877 by comparing K-877-treated wild-type and *Ppara*-null mouse livers using microarrays (Table 5). Probe sets reacting with 753 and 768 were up- or downregulated by K-877 treatment, while few changes (5 and 3 probe sets) were observed in the K-877-treated *Ppara*-null mice. These results clearly indicate that PPAR α is crucial for the regulation of the gene expression by K-877 in the mouse liver. On the other hand, the *Creb3l3* and *Gsta2* mRNA expression levels were up- and downregulated, respectively, by K-877 treatment, even in the absence of the PPAR α (Supplementary Fig.2). These results indicate that the regulation of these two genes by K-877 appears to occur in a PPAR α -independent manner.

Fatty Acid Metabolism is Conserved K-877 Target in Humans and Mice

An overall microarray analysis was performed on K-877-treated primary human hepatocytes and mouse liver tissues. K-877 treatment at a dose of 10 mg/kg altered 1,586 probe sets (Supplementary Table 1) in the mouse liver and 64 mRNA probes in the primary human hepatocytes treated with 10 μ M of K-877. To identify the genes commonly or species specifically regulated by PPAR α agonism in one particular species and clarify genes preferentially regulated by K-877 versus fenofibrate (or fenofibric acid) treatment, a cluster analysis was carried out for a total of 908 genes, excepting genes duplicated or not expressed in one species (Supplementary Fig.3; See Supplementary materials.). Commonly and species specifically regulated genes were identified. Notably, commonly regulated genes included those encoding fatty acid oxidation enzymes (*ACSL1*, *SLC25A20*, *CPT2*, *HADHA*, *HADHB*, *ETFDH*, *ACADVL*, *CPT1A*, *ACAA2*, *ACSL5*), a ketogenic enzyme (*HMGCS2*) and lipid transporters (*CD36*, *VLDLR*, *ABCA1*) (Fig.2). These results support the important conserved role of PPAR α in fatty acid metabolism. Among these commonly regulated genes, *VLDLR*, *FGF21* and *ABCA1* were more highly induced by K-877 than by fenofibric

acid (Fig.3). Because these genes are involved in lipid and carbohydrate metabolism, K-877 may have more favorable effects on metabolism in humans than fenofibrate.

Effect of K-877 on the Peroxisome Proliferation-Related Gene Expression

It is well documented that fibrates induce peroxisome proliferation and subsequently cause hepatocarcinogenesis in rodents²⁸). To ask whether K-877 alters peroxisome β -oxidation and biogenesis, a gene expression analysis was conducted. As a result, K-877 and fenofibrate dramatically induced *Acox1*, *Acots*²⁹) and *Pexs*³⁰) mRNAs in the mouse liver, each of which encode rate-limiting enzymes in peroxisomal fatty acid oxidation, peroxisomal acyl-CoA oxidase, thiolase and peroxisomal biogenesis factor, respectively (Supplementary Fig.4). The induction of these genes reached a maximal level with 1 mg/kg of K-877 treatment. In contrast, 10 μ M K-877 slightly but significantly induced *ACOX1* gene expression, other peroxisomal enzyme and peroxisomal biogenesis factor genes (*PEX1*, *PEX3*, and *PEX11a*); expression of the corresponding human mRNAs were not affected in primary human hepatocytes (Fig.4). Although *ACOX1* induction causes hydrogen peroxide generation, K-877 simultaneously induces the catalase (*CAT*) expression (Supplementary Fig.5) a scavenger of hydrogen peroxide. Therefore, these results suggest that the clinical dose of K-877 may not cause peroxisome proliferation or related liver toxicities in humans.

K-877 Regulates the Gene Expression as a SPPARM α

To examine the influence of different PPAR α agonists on the liver gene expression, the profiles of genes regulated by K-877 and fenofibrate were compared. Up- or downregulated gene mRNAs in the mouse liver were highly comparable between the K-877 and fenofibrate treatment groups (Supplementary Fig.6; See Supplementary materials. and 7). Although no clear differences were observed for the downregulated gene profiles between K-877 and fenofibrate treatment (Supplementary Fig.7; See Supplementary materials.), some K-877 differentially upregulated genes were noted. These included the *Palb2*, *Pla2g7*, *Anxa2*, *Cyp2b9* and *Ndr1* genes, while *Cyp2b10* was specifically upregulated by fenofibrate treatment (Fig.5). Furthermore, a number of species-specific genes regulated by K-877 treatment were observed, including human-specific (e.g. *MBL2* and *ENPEP*) and mouse-specific (e.g. *Ndr1*, *Palb2* and *Pla2g7*) genes (Supplementary Fig.5). Importantly, the *MBL2* and *ENPEP* expression levels were more highly induced by K-877 treatment than by fenofibric acid treatment (Fig.6). Because these genes are involved in innate immunity and blood pressure regulation, K-877 may have additional positive effects other than TG-lowering and HDL-cholesterol-elevating effects in humans. Furthermore, these observations suggest that K-877 and fenofibrate have different modes of target gene regulation. Because the different affinity and structure of PPAR α activators may induce the differential recruitment of co-factors resulting in subtle differences in gene regulation, the present results indicate that K-877 may be classified as a novel SPPARM α .

Discussion

Fibrates are widely prescribed lipid-lowering drugs used to prevent cardiovascular disorders. Clinical studies have clearly demonstrated that fibrate treatment results in a decrease in the plasma TG level and increase in the high-density lipoprotein (HDL)-cholesterol level. The

mechanisms underlying the plasma TG-lowering effect are mainly explained by the induction of TG-rich lipoproteins (TRLs), such as chylomicron, as well as very low-density lipoprotein (VLDL) catabolism and the inhibition of VLDL secretion. These effects are clearly explained by the PPAR α activation induced by fibrates drugs. In fact, numerous reports have demonstrated that PPAR α activation upregulates TG hydrolysis (LPL), fatty acid uptake (FAT/CD36, FATP), fatty acid β -oxidation enzyme production (ACS, CPT-1, HADHA, HADHB) and the ketogenesis (HMGCS2)-related enzyme expression^{31–34} in the mouse liver. Therefore, PPAR α is pivotal for controlling energy homeostasis in mice. However, whether human exposure to PPAR α agonists induces the same key target genes as those found in mice is still a matter of debate. In the current study, there were several notable findings. First, increased fatty acid metabolism induced by K-877 was conserved in the human and mouse liver samples. Second, the activation of PPAR α by K-877 was found to differentially regulate various genes in humans and mice. Third, K-877 and fenofibrate differentially regulated the expression of several target genes via PPAR α activation.

Although weak but consistent with mouse liver microarray data, K-877 and fenofibric acid treatment resulted in increase in fatty acid uptake (*CD36/FAT*), fatty acid β -oxidative genes (*ACSLs*, *CPT1A*, *CPT2*, *ACADVL*, *HADHA*, *HADHB*, *ACAA2*) expression in human hepatocytes. Hence, PPAR α has a conserved function in fatty acid metabolism in both mice and humans, and K-877 and fenofibrate promote the amelioration of dyslipidemia mainly through PPAR α activation. Interestingly, K-877 and fenofibric acid most profoundly induced the *HMGCS2* gene expression in the primary human hepatocytes. Recently, gain- and loss-of function studies have indicated that the HMGCS2 expression is both necessary and sufficient to regulate fatty acid oxidation in HepG2 cells³⁵. This was clearly shown by the fact that PPAR α -induced fatty acid β -oxidation was totally abolished by shRNA knockdown of HMGCS2. Furthermore, the current report showed that the HMGCS2 activity or acetoacetate, the oxidized form of ketone bodies, induced the expression of *FGF21* (another K-877 target gene) via a SirT-1-dependent mechanism. Therefore, these results suggest that HMGCS2 is a key target gene facilitating fatty acid β -oxidation in the human liver. In addition, consistent with the K-877-treated mouse liver microarray data, the robust induction of *PDK4* by K-877 indicated the inactivation of PDH and glucose oxidation in the primary human hepatocytes. These observations indicate that PPAR α agonism by K-877 treatment facilitates mitochondrial long-chain fatty acid β -oxidation through the induction of two key target genes, namely *HMGCS2* and *PDK4*, which change acyl-CoA flux for use in ketogenesis in human hepatocytes.

Among the genes commonly regulated in humans and mice, K-877 effectively induced the *VLDLR*, *FGF21* and *ABCA1* expression compared with fenofibrate. VLDLR is a member of the LDL receptor family mediating the VLDL uptake by peripheral tissues through LPL-dependent lipolysis or receptor-mediated endocytosis³⁶. Therefore, VLDLR plays an important role in VLDL catabolism in many tissues, including skeletal muscle, cardiac and adipose tissues. Recently, Gao *et al.*³⁷ reported that the liver VLDLR expression is upregulated by fenofibrate in a PPAR α -dependent manner and that the TG-lowering effect of fenofibrate is not observed in *Vldl*-null mice. Therefore, K-877 has a greater potential to lower the plasma TG levels than fenofibrate via VLDLR-mediated peripheral VLDL clearance. FGF21 is a member of the fibroblast growth factor family and associated with

mitosis, development, transformation, angiogenesis and survival, having been reported to be a potent metabolic regulator³⁸). Several reports have indicated that FGF21 reduces the fasting plasma glucose, TG, insulin and glucagon levels in diabetic rhesus monkeys, indicating the potential for efficacy in humans^{39, 40}). Although FGF21 has been reported to be a direct PPAR α target gene^{41, 42}), recent work indicates that CREBH⁴³) and HMGCS2³⁵) are also involved in the FGF21 gene expression. In this study, *CREBH* (Supplementary Fig.2) and *HMGCS2* (Fig.2) were induced by K-877. These results imply cooperative mechanisms, such that the combination of PPAR α , CREBH and HMGCS2 is involved in the regulation of the *FGF21* gene expression. In addition, a phase II study showed that 12-week K-877 treatment resulted in increased fasting and postprandial FGF21 levels with an improvement in dyslipidemia^{4, 44}). Importantly, whereas K-877 and fenofibrate show almost the same capacity for plasma TG lowering, K-877 treatment increases the plasma FGF21 level more so than fenofibrate. Although the precise mechanism underlying the K-877-induced *FGF21* gene expression has not been fully clarified, this effect may lead to the beneficial pharmacological effects of K-877 against metabolic disorders.

The *ABCA1* gene is a member of the superfamily of ATP-binding cassette (ABC) transporters that facilitates cellular cholesterol efflux to lipid-poor apoAI and plays a key role in the formation and function of HDL⁴⁵). *ABCA1* is also crucial for reverse cholesterol transport (RCT), and recent data have revealed that it also functions as an anti-inflammatory receptor to suppress the expression of pro-inflammatory factors⁴⁶). K-877 dose-dependently induces the *ABCA1* expression, suggesting the possible beneficial effects of the drug on HDL-cholesterol, inflammation and atherosclerosis.

In contrast, several genes were specifically induced by K-877 treatment in the human hepatocytes and mouse liver tissues. Species differences have been well documented for the peroxisome proliferative response to PPAR α agonists²⁸). Although K-877 robustly induces genes involved in peroxisomal fatty acid β -oxidation in the mouse liver, these genes were not affected in human hepatocytes. To explain this species difference, several mechanisms can be proposed. These include the differences in the PPAR α expression levels, differences in ligand affinity between rats/mice and human PPAR α and species differences in the PPRES of critical target genes⁴⁷). It has been reported that the PPAR α mRNA levels and functional DNA-binding capacity of PPAR α in the human liver are less than those observed in the mouse liver, suggesting that the PPAR α signaling potential is much higher in mice than in humans⁴⁸). However, a number of genes (e.g. *HMGCS2*, *CPT1A*, *ABCA1*) were more highly induced in human hepatocytes than in the mouse liver in the current study. In addition, PPAR α -humanized mice clearly respond to PPAR α agonists in terms of serum triglyceride-lowering effects and the induction of genes encoding fatty acid β -oxidation enzymes, although these mice are resistant to peroxisome proliferation and hepatocellular carcinogenesis. Therefore, the current results suggest that PPAR α -dependent gene regulation is not totally conserved between humans and mice and that the mechanisms underlying species differences should be determined by identifying direct binding sites for PPAR α in individual target genes.

A number of genes were specifically regulated by K-877 treatment in human hepatocytes in the present study. These include mannose-binding lectin 2 (*MBL2*)^{49, 50}) and glutamyl

aminopeptidase *ENPEP*^{51, 52}). MBL is an important protein of the humoral innate immune system, and it has been reported that the serum MBL concentrations are decreased in obese subjects, accompanied by an increase in inflammatory markers⁴⁹). In addition, *G54D MBL2* gene polymorphisms confer an increased risk for developing gestational diabetes mellitus⁵⁰). *ENPEP* is an enzyme that facilitates the conversion of angiotensin II, the main effector protein of the renin-angiotensin-aldosterone system, to angiotensin III⁵¹). In addition, *Enpep* KO mice develop hypertension, and recombinant *ENPEP* treatment significantly decreases systolic blood pressure⁵²), suggesting that *ENPEP* is an essential enzyme for controlling blood pressure. Interestingly, these two genes were dose-dependently upregulated in the primary human hepatocytes, but oppositely downregulated in the mouse liver by K-877. These results suggest that K-877 treatment may improve hypertension, inflammation and associated metabolic disorders.

While a large portion of K-877-regulated genes were found to be regulated in a *PPARα*-dependent manner, the *Creb3l3* and *Gsta2* genes expression levels were up- and downregulated, respectively, by K-877 treatment, even in the absence of the *PPARα*. *Creb3l3* (CREBH) is a transcription factor belonging to the cyclic AMP response element binding protein transcription factor (CREB/ATF) family⁵³) and has been reported to regulate the serum amyloid P-component (*SAP*) and C-reactive protein (*CRP*) gene expression in response to systemic inflammatory signaling in the liver⁵⁴). In addition, *CREB3l3* was shown to regulate gluconeogenesis via the modulation of the *Pepck-c* and *G6Pase* genes⁵⁵) as well as many genes involved in hepatic lipid metabolism⁵⁶). Furthermore, others have suggested that *Creb3l3* is a direct target of *PPARα* and that the induction of *CREB3l3* is involved in nutritional regulation in the fasting state⁵⁷). On the other hand, a reduced expression of glutathione S-transferase A2 (*GSTA2*), which catalyzes the reduction of reactive oxygen species through glutathione (GSH) utilization, has been shown to be involved in *PPARα* agonist-induced hepatocellular carcinogenesis⁵⁸). Moreover, it has been shown that the *Gsta2* expression is transcriptionally regulated by *NRF2* and *C/EBPβ*. In addition, functional multiple *PPRE*-responsive enhancer module (*PPREM*) was recently reported in the *Gsta2* promoter, by which *PPARγ* agonists induce the *Gsta2* expression via *PPREM* in hepatocytes⁵⁹). Therefore, the mechanism underlying the reduction of the *Gsta2* expression induced by K-877 treatment in *Ppara*-null mice has not yet been established. In addition, neither inflammation-related genes (*SAP* and *CRP*) nor genes involved in gluconeogenesis and lipid metabolism were affected by K-877 treatment in the *Ppara*-null mice. Although these observations may suggest the low contribution of *CREB3l3* induction to the pharmacological effects of K-877, the possibility of a cooperative effect of *CREB3l3* and other transcription factors (i.e. *PPARα*) in controlling the target gene expression cannot be ruled out. In fact, it was recently reported that the active form of *CREB3l3* interacts with *PPARα* to form a functional complex that synergistically induces the *Fgf21* gene expression in the fasting state⁶⁰). Hence, further investigations are needed to understand the precise mechanisms underlying the transcriptional regulation of the *Creb3l3* and *Gsta2* genes.

Conclusion

In conclusion, K-877 regulates the gene expression mainly through *PPARα* activation. Regulation of the fatty acid β -oxidative gene expression by K-877 is well conserved

between human hepatocytes and the mouse liver. K-877 regulates clinically beneficial target genes (*VLDLR*, *FGF21*, *ABCA1*, *MBL2*, *ENPEP*) and thus may be superior to other fibrate drugs on the market.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by a grant for Translational Systems Biology and Medicine Initiative from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- 1). Hotamisligil GS: Inflammation and metabolic disorders. *Nature*, 2006; 444: 860–867 [PubMed: 17167474]
- 2). Wierzbicki AS: Fibrates after the FIELD study: Some answers, more questions. *Diab Vasc Dis Res*, 2006; 3: 166–171 [PubMed: 17160911]
- 3). Fruchart JC: Peroxisome proliferator-activated receptor- α (PPAR α): at the crossroads of obesity, diabetes and cardiovascular disease. *Atherosclerosis*, 2009; 205: 1–8 [PubMed: 19386311]
- 4). Fruchart JC: Selective peroxisome proliferator-activated receptor α modulators (SPPARM α): the next generation of peroxisome proliferator-activated receptor α -agonists. *Cardiovasc Diabetol*, 2013; 12: 82 [PubMed: 23721199]
- 5). Yamazaki Y, Abe K, Toma T, Nishikawa M, Ozawa H, Okuda A, Araki T, Oda S, Inoue K, Shibuya K, Staels B, Fruchart JC: Design and synthesis of highly potent and selective human peroxisome proliferator-activated receptor α agonists. *Bioorg Med Chem Lett*, 2007; 17: 4689–4693 [PubMed: 17553678]
- 6). Ishibashi S, Ymashita S, Arai H, Araki E, Yokote K, Kodama T: Efficacy and safety of K-877, a potent and selective PPAR- α agonist, in Japanese patients with dyslipidemia. *American Heart Association, Scientific sessions 2013*, 2013; poster 2002
- 7). Oliver WR Jr, Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, Xu HE, Sternbach DD, Kliewer SA, Hansen BC, Willson TM: A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci USA*, 2001; 98: 5306–5311 [PubMed: 11309497]
- 8). Tanaka T, Yamamoto J, Iwasaki S, Asaba H, Hamura H, Ikeda Y, Watanabe M, Magoori K, Ioka RX, Tachibana K, Watanabe Y, Uchiyama Y, Sumi K, Iguchi H, Ito S, Doi T, Hamakubo T, Naito M, Auwerx J, Yanagisawa M, Kodama T, Sakai J: Activation of peroxisome proliferator-activated receptor δ induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci USA*, 2003; 100: 15924–15929 [PubMed: 14676330]
- 9). Akita N, Tsujita M, Yokota T, Gonzalez FJ, Ohte N, Kimura G, Yokoyama S: High density lipoprotein turnover is dependent on peroxisome proliferator-activated receptor α in mice. *J Atheroscler Thromb*, 2010; 17: 1149–1159 [PubMed: 20668363]
- 10). Tanaka T, Tahara-Hanaoka S, Nabekura T, Ikeda K, Jiang S, Tsutsumi S, Inagaki T, Magoori K, Higurashi T, Takahashi H, Tachibana K, Tsurutani Y, Raza S, Anai M, Minami T, Wada Y, Yokote K, Doi T, Hamakubo T, Auwerx J, Gonzalez FJ, Nakajima A, Aburatani H, Naito M, Shibuya A, Kodama T, Sakai J: PPAR β/δ activation of CD300a controls intestinal immunity. *Sci Rep*, 2014; 4: 5412 [PubMed: 24958459]
- 11). Rodríguez JC, Gil-Gómez G, Hegardt FG, Haro D: Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. *J Biol Chem*, 1994; 269: 18767–18772 [PubMed: 7913466]

- 12). Wu P, Peters JM, Harris RA: Adaptive increase in pyruvate dehydrogenase kinase 4 during starvation is mediated by peroxisome proliferator-activated receptor α . *Biochem Biophys Res Commun*, 2001; 287: 391–396 [PubMed: 11554740]
- 13). Martin G, Poirier H, Hennuyer N, Crombie D, Fruchart JC, Heyman RA, Besnard P, Auwerx J: Induction of the fatty acid transport protein 1 and acyl-CoA synthase genes by dimer-selective rexinoids suggests that the peroxisome proliferator-activated receptor-retinoid X receptor heterodimer is their molecular target. *J Biol Chem*, 2000; 275: 12612–12618 [PubMed: 10777552]
- 14). Huang B, Wu P, Bowker-Kinley MM, Harris RA: Regulation of pyruvate dehydrogenase kinase expression by peroxisome proliferator-activated receptor- α ligands, glucocorticoids, and insulin. *Diabetes*, 2002; 51: 276–283 [PubMed: 11812733]
- 15). Kersten S: Integrated physiology and systems biology of PPAR α . *Mol Metab*, 2014; 3: 354–371 [PubMed: 24944896]
- 16). Dalen KT, Ulven SM, Arntsen BM, Solaas K, Nebb HI: PPAR α activators and fasting induce the expression of adipose differentiation-related protein in liver. *J Lipid Res*, 2006; 47: 931–943 [PubMed: 16489205]
- 17). Nourbakhsh M, Douglas DN, Pu CH, Lewis JT, Kawahara T, Lisboa LF, Wei E, Asthana S, Quiroga AD, Law LM, Chen C, Addison WR, Nelson R, Houghton M, Lehner R, Kneteman NM: Arylacetamide deacetylase: a novel host factor with important roles in the lipolysis of cellular triacylglycerol stores, VLDL assembly and HCV production. *J Hepatol*, 2013; 59: 336–343 [PubMed: 23542347]
- 18). Fiermonte G, Paradies E, Todisco S, Marobbio CM, Palmieri F: A novel member of solute carrier family 25 (SLC25A42) is a transporter of coenzyme A and adenosine 3',5'-diphosphate in human mitochondria. *J Biol Chem*, 2009; 284: 18152–18159 [PubMed: 19429682]
- 19). Bumpus NN, Johnson EF: 5-Aminoimidazole-4-carboxamide-ribonucleoside (AICAR)-stimulated hepatic expression of Cyp4a10, Cyp4a14, Cyp4a31, and other peroxisome proliferator-activated receptor α -responsive mouse genes is AICAR 5'-monophosphate-dependent and AMP-activated protein kinase-independent. *J Pharmacol Exp Ther*, 2011; 339: 886–895 [PubMed: 21896918]
- 20). Kim YJ, Cho SY, Yun CH, Moon YS, Lee TR, Kim SH: Transcriptional activation of Cidec by PPAR γ 2 in adipocyte. *Biochem Biophys Res Commun*, 2008; 377: 297–302 [PubMed: 18845124]
- 21). Dongol B, Shah Y, Kim I, Gonzalez FJ, Hunt MC: The acyl-CoA thioesterase I is regulated by PPAR α and HNF4 α via a distal response element in the promoter. *J Lipid Res*, 2007; 48: 1781–1791 [PubMed: 17485727]
- 22). Hunt MC, Lindquist PJ, Nousiainen S, Huttunen M, Orii K, Svensson TL, Aoyama T, Hashimoto T, Diczfalussy U, Alexson SE: Acyl-CoA thioesterases belong to a novel gene family of peroxisome proliferator-regulated enzymes involved in lipid metabolism. *Cell Biochem Biophys*, 2000; 32: 317–324 [PubMed: 11330065]
- 23). Hunt MC, Nousiainen SE, Huttunen MK, Orii KE, Svensson LT, Alexson SE: Peroxisome proliferator-induced long chain acyl-CoA thioesterases comprise a highly conserved novel multi-gene family involved in lipid metabolism. *J Biol Chem*, 1999; 274: 34317–34326 [PubMed: 10567408]
- 24). Rommelaere S, Millet V, Gensollen T, Bourges C, Eeckhoutte J, Hennuyer N, Baugé E, Chasson L, Cacciatore I, Staels B, Pitari G, Galland F, Naquet P: PPAR α regulates the production of serum Vanin-1 by liver. *FEBS Lett*, 2013; 587: 3742–3748 [PubMed: 24140347]
- 25). van Diepen JA, Jansen PA, Ballak DB, Hijmans A, Hooiveld GJ, Rommelaere S, Galland F, Naquet P, Rutjes FP, Mensink RP, Schrauwen P, Tack CJ, Netea MG, Kersten S, Schalkwijk J, Stienstra R: PPAR- α dependent regulation of vanin-1 mediates hepatic lipid metabolism. *J Hepatol*, 2014; 61: 366–372 [PubMed: 24751833]
- 26). Jiang YJ, Kim P, Lu YF, Feingold KR: PPAR γ activators stimulate aquaporin 3 expression in keratinocytes/epidermis. *Exp Dermatol*. 2011; 20: 595–599 [PubMed: 21457357]
- 27). Jancova P, Anzenbacher P, Anzenbacherova E: Phase II drug metabolizing enzymes. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 2010; 154: 103–116 [PubMed: 20668491]

- 28). Gonzalez FJ, Shah YM: PPAR α : Mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology*, 2008; 246: 2–8 [PubMed: 18006136]
- 29). Hunt MC, Tillander V, Alexson SE: Regulation of peroxisomal lipid metabolism: the role of acyl-CoA and coenzyme A metabolizing enzymes. *Biochimie*, 2014; 98: 45–55 [PubMed: 24389458]
- 30). Steinberg SJ, Dodt G, Raymond GV, Braverman NE, Moser AB, Moser HW: Peroxisome biogenesis disorders. *Biochim Biophys Acta*, 2006; 1763: 1733–1748 [PubMed: 17055079]
- 31). Schoonjans K, Staels B, Auwerx J: Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res*, 1996; 37: 907–925 [PubMed: 8725145]
- 32). Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, Grimaldi PA, Kadowaki T, Lazar MA, O'Rahilly S, Palmer CN, Plutzky J, Reddy JK, Spiegelman BM, Staels B, Wahli W: International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacol Rev*, 2006; 58: 726–741 [PubMed: 17132851]
- 33). Lefebvre P, Chinetti G, Fruchart JC, Staels B: Sorting out the roles of PPAR α in energy metabolism and vascular homeostasis. *J Clin Invest*, 2006; 116: 571–580 [PubMed: 16511589]
- 34). Miyazaki M, Nakagawa I, Koga S, Kasahara Y, Patricelli MP: Proteomics analysis of cardiac muscle from rats with peroxisomal proliferator-activated receptor α (PPAR α) stimulation. *J Toxicol Sci*, 2010; 35: 131–135 [PubMed: 20118634]
- 35). Vilà-Brau A, De Sousa-Coelho AL, Mayordomo C, Haro D, Marrero PF: Human HMGCS2 regulates mitochondrial fatty acid oxidation and FGF21 expression in HepG2 cell line. *J Biol Chem*, 2011; 286: 20423–20430 [PubMed: 21502324]
- 36). Webb JC, Patel DD, Jones MD, Knight BL, Soutar AK: Characterization and tissue-specific expression of the human 'very low density lipoprotein (VLDL) receptor' mRNA. *Hum Mol Genet*, 1994; 3: 531–537 [PubMed: 8069294]
- 37). Gao Y, Shen W, Zhang Q, Hu Y, Chen Y: Up-regulation of Hepatic VLDLR via PPAR α Is Required for the Triglyceride-Lowering Effect of Fenofibrate. *J Lipid Res*, 2014; 55: 1622–1633 [PubMed: 24899625]
- 38). Li H, Zhang J, Jia W: Fibroblast growth factor 21: a novel metabolic regulator from pharmacology to physiology. *Front Med*, 2013; 7: 25 [PubMed: 23358894]
- 39). Kharitonov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB: FGF-21 as a novel metabolic regulator. *J Clin Invest*, 2005; 115: 1627–1635 [PubMed: 15902306]
- 40). Véniant MM, Komorowski R, Chen P, Stanislaus S, Winters K, Hager T, Zhou L, Wada R, Hecht R, Xu J: Long-acting FGF21 has enhanced efficacy in diet-induced obese mice and in obese rhesus monkeys. *Endocrinology*, 2012; 153: 4192–4203 [PubMed: 22798348]
- 41). Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, Kliewer SA: Endocrine regulation of the fasting response by PPAR α -mediated induction of fibroblast growth factor 21. *Cell Metab*, 2007; 5: 415–425 [PubMed: 17550777]
- 42). Lundåsen T, Hunt MC, Nilsson LM, Sanyal S, Angelin B, Alexson SE, Rudling M: PPAR α is a key regulator of hepatic FGF21. *Biochem Biophys Res Commun*, 2007; 360: 437–440 [PubMed: 17601491]
- 43). Kim H, Mendez R, Zheng Z, Chang L, Cai J, Zhang R, Zhang K: Liver-enriched transcription factor CREBH interacts with peroxisome proliferator-activated receptor α to regulate metabolic hormone FGF21. *Endocrinology*, 2014; 155: 769–782 [PubMed: 24424044]
- 44). Yokote K, Ishibashi S, Yamashita S, Arai H, Araki E, Kodama T: Marked Increase of Plasma Fibroblast Growth Factor 21 in Dyslipidemic Patients Treated With K-877, a Novel Highly Potent and Specific Peroxisome Proliferator-Activated Receptor Alpha Agonist. *American Heart Association Scientific Sessions 2013*, 2013; Abstract 17336
- 45). Van Eck M: ATP-binding cassette transporter A1: key player in cardiovascular and metabolic disease at local and systemic level. *Curr Opin Lipidol*, 2014; 25: 297–303 [PubMed: 24992457]

- 46). Yin K, Liao DF, Tang CK: ATP-binding membrane cassette transporter A1 (ABCA1): a possible link between inflammation and reverse cholesterol transport. *Mol Med*, 2010; 16: 438–449 [PubMed: 20485864]
- 47). Ammerschlaeger M, Beigel J, Klein KU, Mueller SO: Characterization of the species-specificity of peroxisome proliferators in rat and human hepatocytes. *Toxicol Sci*, 2004; 78: 229–240 [PubMed: 14976357]
- 48). Palmer CN, Hsu MH, Griffin KJ, Raucy JL, Johnson EF: Peroxisome proliferator activated receptor- α expression in human liver. *Mol Pharmacol*, 1998; 53: 14–22 [PubMed: 9443928]
- 49). Ip WK, Takahashi K, Ezekowitz RA, Stuart LM: Mannose-binding lectin and innate immunity. *Immunol Rev*, 2009; 230: 9–21 [PubMed: 19594626]
- 50). Megia A, Gallart L, Fernández-Real JM, Vendrell J, Simón I, Gutierrez C, Richart C: Mannose-binding lectin gene polymorphisms are associated with gestational diabetes mellitus. *J Clin Endocrinol Metab*, 2004; 89: 5081–5087 [PubMed: 15472209]
- 51). Mizutani S, Ishii M, Hattori A, Nomura S, Numaguchi Y, Tsujimoto M, Kobayashi H, Murohara T, Wright JW: New insights into the importance of aminopeptidase A in hypertension. *Heart Fail Rev*, 2008; 13: 273–284 [PubMed: 17990103]
- 52). Mitsui T, Nomura S, Okada M, Ohno Y, Kobayashi H, Nakashima Y, Murata Y, Takeuchi M, Kuno N, Nagasaka T, O-Wang J, Cooper MD, Mizutani S: Hypertension and angiotensin II hypersensitivity in aminopeptidase A-deficient mice. *Mol Med*, 2003; 9: 57–62 [PubMed: 12765341]
- 53). Omori Y, Imai J, Watanabe M, Komatsu T, Suzuki Y, Kataoka K, Watanabe S, Tanigami A, Sugano S: CREB-H: a novel mammalian transcription factor belonging to the CREB/ATF family and functioning via the box-B element with a liver-specific expression. *Nucleic Acids Res*, 2001; 29: 2154–2162 [PubMed: 11353085]
- 54). Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, Back SH, Kaufman RJ: Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell*, 2006; 124: 587–599 [PubMed: 16469704]
- 55). Lee MW, Chanda D, Yang J, Oh H, Kim SS, Yoon YS, Hong S, Park KG, Lee IK, Choi CS, Hanson RW, Choi HS, Koo SH: Regulation of hepatic gluconeogenesis by an ER-bound transcription factor, CREBH. *Cell Metab*, 2010; 11: 331–339 [PubMed: 20374965]
- 56). Zhang C, Wang G, Zheng Z, Maddipati KR, Zhang X, Dyson G, Williams P, Duncan SA, Kaufman RJ, Zhang K: Endoplasmic reticulum-tethered transcription factor cAMP responsive element-binding protein, hepatocyte specific, regulates hepatic lipogenesis, fatty acid oxidation, and lipolysis upon metabolic stress in mice. *Hepatology*, 2012; 55: 1070–1082 [PubMed: 22095841]
- 57). Danno H, Ishii KA, Nakagawa Y, Mikami M, Yamamoto T, Yabe S, Furusawa M, Kumadaki S, Watanabe K, Shimizu H, Matsuzaka T, Kobayashi K, Takahashi A, Yatoh S, Suzuki H, Yamada N, Shimano H: The liver-enriched transcription factor CREBH is nutritionally regulated and activated by fatty acids and PPAR α . *Biochem Biophys Res Commun*, 2010; 391: 1222–1227 [PubMed: 20006574]
- 58). Nishimura J, Dewa Y, Okamura T, Muguruma M, Jin M, Saegusa Y, Umemura T, Mitsumori K: Possible involvement of oxidative stress in fenofibrate-induced hepatocarcinogenesis in rats. *Arch Toxicol*, 2008; 82: 641–654 [PubMed: 18253720]
- 59). Park EY, Cho IJ, Kim SG: Transactivation of the PPAR-responsive enhancer module in chemopreventive glutathione S-transferase gene by the peroxisome proliferator-activated receptor- γ and retinoid X receptor heterodimer. *Cancer Res*, 2004; 64: 3701–3713 [PubMed: 15150131]
- 60). Kim H, Mendez R, Zheng Z, Chang L, Cai J, Zhang R, Zhang K: Liver-enriched transcription factor CREBH interacts with peroxisome proliferator-activated receptor α to regulate metabolic hormone FGF21. *Endocrinology*, 2014; 155: 769–782 [PubMed: 24424044]

Fig.1.

K-877 is a selective PPAR α modulator. Dose-response curves are shown for human PPAR α , human PPAR δ and human PPAR γ transactivation. (a) CHO cell-based transactivation assay. (b) HepG2 cell-based transactivation assay.

Fig.2.

Heat map illustrating the conserved genes induced by K-877 treatment in the mouse liver and primary human hepatocytes.

Fig.3.

K-877 effectively induces the *VLDLR*, *FGF21* and *ABCA1* mRNA expression in primary human hepatocytes. The data represent $\pm$ s.e.m. * p <0.05; ** p <0.01.

Fig.4.

Effect of K-877 on the peroxisomal β -oxidation and biogenesis-related gene expression. The data represent $\pm$ s.e.m. * $p < 0.05$; ** $p < 0.01$.

Fig.5.

Genes differentially regulated by the PPAR α agonist. Effects of K-877 and fenofibrate on the differentially regulated gene expression. The data represent $\pm$ s.e.m. * p <0.05; ** p <0.01.

Fig.6.

Effect of K-877 on the species-specific target gene expression. The data represent $\pm$ s.e.m.

* $p < 0.05$; ** $p < 0.01$.

Table 1. Characteristics of the primary human hepatocytes and response to K-877 and fenofibric acid

Donor	EJW	JGM	DOO
Gender	female	female	male
Age	29	54	57
Height	66"	62"	72"
Weight	85 kg	74 kg	85 kg
Alcohol	○	×	×
Tobacco	○	×	×
Drug	○	×	×
DM	T1D	T2D	T2D
Cell shape	Distorted	Round	Distorted
Cell size	Large	Small	Large
Viability	88.1%	83.8%	76.5%
Expressed genes	11935	12234	11936
100 nM K-877 treatment			
Up-regulated probe sets	81	23	102
Down-regulated probe sets	58	14	48
10 μ M K-877 treatment			
Up-regulated probe sets	235	134	352
Down-regulated probe sets	115	52	116
100 μ M fenofibric acid treatment			
Up-regulated probe sets	72	27	126
Down-regulated probe sets	98	28	64

Hepatocytes were treated with DMSO or K-877 and fenofibrate as described in the Materials & Methods. The average difference is normalized to 100 in each experiment. The criterion for a significant induction in a particular gene was an average difference equal to or more than 100 for the K-877- or fenofibric acid-treated primary human hepatocytes and a fold change equal to or greater than 2^{0.6}. The criterion for a significant reduction in a particular gene was an average difference equal to or more than 100 for the DMSO-treated primary human hepatocytes and a fold change equal to or less than 2^{-0.6}.

Table 2. Top 20 genes most profoundly induced in the primary human hepatocytes treated with K-877

	100 nM K-877			10 μ M K-877			100 μ M FEN		
	EJW	JGM	DOO	EJW	JGM	DOO	EJW	JGM	DOO
<u>HMGC2</u>	3.2	2.0	4.1	5.3	3.9	5.2	3.3	1.6	3.7
<u>FABP1</u>	1.6	0.3	2.4	3.6	1.4	4.4	1.5	1.6	2.6
<u>PK4</u>	1.8	1.9	3.1	3.2	3.3	4.6	1.5	1.6	2.6
<u>ANGPTL4</u>	1.0	1.3	1.7	2.1	3.2	2.9	0.9	1.1	1.6
<u>ADFP</u>	1.2	1.2	1.0	1.7	2.4	1.0	0.3	0.4	0.0
<u>TXNIP</u>	0.6	0.6	0.6	1.6	2.3	2.0	0.3	0.4	0.0
<u>AADAC</u>	0.6	0.5	0.7	1.5	1.4	1.4	0.6	0.5	0.1
<u>ATP2B4</u>	0.5	0.2	0.7	1.4	0.9	1.2	0.3	0.4	0.5
<u>SLC25A42</u>	0.5	0.4	0.7	1.4	1.0	1.7	0.4	0.5	0.7
<u>ANXA1</u>	0.3	0.3	0.5	1.3	0.9	1.3	0.4	0.3	0.4
<u>ACSL1</u>	0.5	0.6	0.9	1.2	1.4	1.3	0.6	0.7	0.9
<u>SH3BGRL2</u>	0.4	0.4	0.5	1.2	0.7	0.8	0.5	0.4	0.4
<u>SGK2</u>	0.1	0.1	0.5	1.2	1.0	1.3	0.2	0.3	0.5
<u>AKR1B1</u>	0.1	0.3	0.4	1.1	0.9	1.2	0.3	0.1	0.3
<u>CPT1A</u>	0.6	0.6	0.8	1.1	1.3	1.4	0.7	0.6	0.8
<u>FSTL1</u>	0.3	0.3	0.4	1.1	0.9	1.2	0.3	0.5	0.5
<u>HSDL2</u>	0.5	0.3	0.7	1.1	0.8	1.4	0.6	0.2	0.7
<u>VLDLR</u>	0.2	0.2	0.1	1.0	0.9	0.9	0.2	0.2	0.2
<u>CDK3</u>	0.2	0.1	0.3	1.0	0.8	1.3	0.1	0.1	0.2
<u>SLC25A20</u>	0.3	0.6	0.6	0.9	1.0	1.1	0.5	0.6	0.6

The data are presented as the fold change (2^n) compared to the DMSO-treated primary human hepatocytes. Underlined gene probes are involved in carbohydrate or lipid metabolism.

Table 3.

Top 20 genes most profoundly induced in the mouse liver treated with K-877 for three days

Gene symbol or probe number	1 mg/kg K-877			3 mg/kg K-877			10 mg/kg K-877			100 mg/kg FEN		
	1	2	3	1	2	3	1	2	3	1	2	3
<u>Cyp4a3l</u>	7.2	6.9	6.9	7.4	7.6	7.1	7.8	7.3	8.0	7.9	7.9	8.1
<u>Pdk4</u>	7.0	6.9	6.9	7.4	7.5	7.2	7.4	7.3	7.1	7.1	6.8	6.5
<u>I446423_at</u>	6.7	6.5	6.6	6.7	6.6	6.5	6.8	6.9	6.6	6.5	6.4	6.4
<u>I443147_at</u>	7.1	6.8	6.9	6.9	6.6	6.9	6.7	7.0	6.8	6.9	7.0	6.8
<u>Rad51l1</u>	5.9	5.7	5.9	5.9	6.1	6.0	6.5	6.8	6.3	6.0	6.2	6.1
<u>Cidec</u>	4.1	3.5	5.1	5.0	6.1	5.3	6.5	6.9	7.5	3.9	4.0	4.1
<u>Acot2</u>	5.3	5.2	5.5	5.7	5.7	5.9	6.2	6.6	6.6	5.9	6.1	6.0
<u>Scnn1g</u>	4.3	4.3	4.4	5.6	5.7	5.0	6.1	6.1	6.2	5.2	5.8	5.2
<u>Acot3</u>	6.1	6.0	6.0	6.0	5.9	6.0	6.0	6.1	5.8	5.9	5.9	5.7
<u>Ly6d</u>	3.4	3.6	4.4	4.5	4.8	4.8	5.8	5.7	6.5	4.2	5.0	4.3
<u>Slc27a1</u>	3.1	3.1	2.5	3.3	4.0	3.6	5.2	5.4	4.3	4.2	3.9	4.2
<u>Gadd45b</u>	4.0	3.2	3.5	4.3	3.7	4.7	5.2	5.0	5.0	4.8	4.2	4.1
<u>Vim1</u>	4.3	4.3	4.4		4.5	4.6	5.0	5.0	4.5	4.4	4.6	4.3
<u>Cox6b2</u>	2.5	2.4	2.3	3.1	3.9	2.9	4.8	4.3	4.1	3.4	3.8	3.6
<u>Acot1</u>	4.4	4.5	4.4	4.4	4.7	4.6	4.8	4.8	4.6	4.6	4.7	4.5
<u>App3</u>	2.9	2.3	2.8	3.7	4.2	3.4	4.8	4.7	4.6	2.5	3.1	3.4
<u>Lamb3</u>	3.0	2.9	3.0	3.3	3.6	3.6	4.7	4.4	4.1	3.1	3.3	2.9
<u>Serpine1</u>	3.6	4.1	4.7	5.1	4.3	5.1	4.7	5.5	4.5	4.6	4.5	4.5
<u>Hsd17b11</u>	3.4	3.3	3.2	3.8	4.1	4.0	4.7	4.7	4.4	4.2	4.4	4.6
<u>Serinc2</u>	3.9	3.8	3.5	3.9	4.3	4.4	4.7	4.9	4.7	3.9	4.3	4.4

The data are presented as the fold change (2ⁿ) compared to the vehicle-treated C57Bl/6J mice. Underlined gene probes are involved in carbohydrate or lipid metabolism.

Table 4.

Top 20 genes most profoundly reduced in the mouse liver treated with K-877 for three days

Gene symbol	1 mg/kg K-877			3 mg/kg K-877			10 mg/kg K-877			100 mg/kg FEN		
	1	2	3	1	2	3	1	2	3	1	2	3
<i>Serpina12</i>	-4.0	-3.8	-4.4	-4.5	-4.3	-5.0	-6.4	-5.2	-5.5	-4.7	-4.2	-5.0
<i>Clec2h</i>	-4.3	-4.3	-4.3	-5.2	-5.8	-5.4	-6.2	-7.2	-6.0	-4.8	-4.8	-4.7
<i>Car3</i>	-2.3	-2.6	-3.8	-3.1	-3.6	-3.6	-4.6	-5.8	-6.5	-2.8	-3.3	-2.1
<i>Hsd3b5</i>	-2.6	-2.1	-2.7	-2.2	-1.6	-2.6	-4.1	-5.7	-8.2	-2.6	-3.0	-2.6
<i>Rgs16</i>	-2.1	-2.7	-2.2	-2.6	-4.2	-4.1	-3.9	-3.4	-2.7	-3.4	-2.6	-4.5
<i>Ppp1r3c</i>	-2.1	-2.2	-3.1	-3.4	-2.9	-3.5	-3.7	-3.5	-4.0	-3.1	-3.2	-2.9
<i>Fam47e</i>	-2.1	-1.7	-1.7	-2.1	-2.4	-2.2	-3.6	-3.6	-5.0	-1.5	-1.4	-1.6
<i>Sleo1a4</i>	-1.3	-1.5	-1.3	-1.9	-3.6	-2.0	-3.5	-4.3	-4.1	-2.6	-3.5	-3.4
<i>Sult1a1</i>	-0.9	-1.1	-1.1	-1.8	-3.6	-1.8	-3.5	-4.3	-4.3	-1.9	-3.0	-2.7
<i>Ardc3</i>	-1.4	-1.7	-1.3	-1.7	-2.5	-2.5	-3.5	-2.8	-2.7	-1.5	-2.3	-2.1
<i>Enho</i>	-1.6	-1.2	-1.4	-1.2	-1.6	-1.8	-3.4	-3.2	-2.6	-2.6	-2.5	-1.8
<i>Inhba</i>	-2.6	-1.5	-1.2	-1.5	-1.2	-1.3	-3.1	-2.3	-1.4	-1.6	-1.8	-1.8
<i>Slc22a7</i>	-2.0	-1.0	-1.7	-1.9	-1.8	-2.5	-3.1	-4.0	-5.4	-2.3	-2.7	-2.4
<i>Cyp2c54</i>	-1.2	-1.3	-1.4	-1.8	-2.8	-1.8	-3.1	-4.0	-5.6	-1.8	-2.6	-2.4
<i>Apo19a/Apo19b</i>	-2.1	-1.7	-2.1	-2.4	-2.2	-1.9	-3.0	-3.9	-3.1	-2.0	-2.1	-2.5
<i>Upp2</i>	-1.6	-1.5	-1.8	-2.3	-3.5	-2.1	-3.0	-3.6	-3.8	-1.5	-1.5	-1.7
<i>BC089597</i>	-1.2	-1.2	-1.1	-1.5	-2.1	-1.6	-3.0	-2.8	-2.5	-1.6	-2.2	-1.9
<i>1700023H06Rik</i>	-2.8	-2.8	-2.8	-2.2	-2.9	-2.9	-3.0	-2.9	-3.3	-2.0	-2.4	-2.2
<i>1459948_at</i>	-2.3	-2.6	-2.0	-2.0	-1.1	-1.0	-3.0	-2.7	-2.9	-2.2	-2.5	-1.9
<i>Kalrn</i>	-1.8	-1.2	-1.9	-2.0	-2.5	-2.9	-2.1	-2.1	-2.1	-2.1	-2.0	-1.7

The data are presented as the fold change (2ⁿ) compared to the vehicle-treated C57Bl/6J mice.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 5.

Microarray analysis of the K-877-treated wild-type and *Ppara*-null mouse liver

3 mg/kg/day K-877		
	vs Wild-type vehicle	vs <i>Ppara</i> -null vehicle
Up regulated probe sets	753	5
Down regulated probe sets	768	3

Mice were treated with vehicle or K-877 as described in the Materials & Methods. The average difference is normalized to 100 in each experiment. The criterion for the significant induction of a particular gene was an average difference equal to or more than 100 for the K-877-treated mouse liver samples and a fold change equal to or greater than $2^{0.6}$. The criterion for a significant reduction in a particular gene was an average difference equal to or more than 100 for the vehicle-treated mouse liver samples and a fold change equal to or less than $2^{-0.6}$.