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PPAR α -dependent increase of mouse urine output by gemfibrozil and fenofibrate

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Abstract

While gemfibrozil and fenofibrate are prescribed for anti-dyslipidemia treatment, a rational basis for the use of these drugs for treatment of dyslipidemia with concurrent metabolic syndrome has not been established. In this study, wild-type and *Ppara*-null mice were fed gemfibrozil- or fenofibrate-containing diets for 14 days. Urine output (24 h) was monitored, and urine, serum, and liver and kidney tissues were subjected to toxicity assessment. A 2-month challenge followed by a 2-week wash-out was performed for gemfibrozil to determine urine output and the potential toxicity. A therapeutically equivalent dose of gemfibrozil was more effective than fenofibrate in increasing urine output. This regulatory effect was not observed in *Ppara*-null mice. In contrast, hepatomegaly induced by fenofibrate was more pronounced than that of gemfibrozil. No significant toxicity was observed in liver or kidney in the 2-month treatment with gemfibrozil. These data demonstrated PPAR mediates the increased urine output by fibrates. Considering the relative action on hepatomegaly and the regulatory effect on urine output, gemfibrozil may be the

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Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

preferable drug to increase urine output. These results revealed a new pharmacodynamic effect of clinically prescribed PPAR α agonists and suggested the potential value of gemfibrozil in modification of blood pressure.

Abstract

Le gemfibrozil et le fénofibrate sont des médicaments prescrits pour traiter les dyslipidémies, mais les fondements de leur utilisation n'ont pas encore été établis dans le cas des dyslipidémies associées au syndrome métabolique. Dans cette étude, nous avons administré pendant 14 jours un régime alimentaire contenant du gemfibrozil ou du fénofibrate à des souris nulles *Ppara* et de type sauvage. Nous avons surveillé le débit urinaire (sur 24 heures) et procédé à des évaluations toxicologiques de l'urine, du sérum et des tissus rénal et hépatique. Afin de déterminer le débit urinaire et une toxicité éventuelle, nous avons administré le gemfibrozil pendant une période de provocation de 2 mois, suivie d'une période de sevrage de 2 semaines. À une dose équivalente à celle qui est utilisée en thérapeutique, le gemfibrozil était plus efficace que le fénofibrate pour augmenter le débit urinaire. Nous n'avons pas observé cet effet régulateur chez la souris nulle *Ppara*. Inversement, l'hépatomégalie engendrée avec le fénofibrate était plus importante qu'avec le gemfibrozil. Nous n'avons observé aucune toxicité hépatique ou rénale notable avec l'administration de gemfibrozil pendant 2 mois. Ces données montraient que le PPAR α assure la médiation de l'augmentation du débit urinaire par les fibrates. Étant donné leurs effets respectifs sur l'hépatomégalie et la régulation du débit urinaire, le gemfibrozil pourrait être un médicament plus avantageux que le fénofibrate pour augmenter le débit urinaire. Ces résultats ont révélé un nouvel effet pharmacodynamique d'agonistes du PPAR α prescrits en clinique et laissaient entrevoir la valeur potentielle du gemfibrozil pour modifier la tension artérielle. [Traduit par la Rédaction]

Keywords

gemfibrozil; urine output; PPAR α ; fenofibrate

Introduction

Metabolic syndrome (MS) is characterized by high serum triglyceride (TG), elevated blood pressure, abdominal obesity, elevated fasting plasma glucose, and low high-density lipoprotein-cholesterol (HDL-C). In the United States, the unadjusted MS prevalence was reported to be 21.8%, which increased to 43.5% and 42.0% for participants aged 60–69 years and those of 70 years and above, respectively (Ford et al. 2002). The Lifestyle Interventions and Independence for Elders Study reported a prevalence of 49.8% in a population of sedentary adults aged 70–89 years (Botosaneanu et al. 2015). Concurrent metabolic diseases trigger more therapeutic challenges for clinicians. Approximately 50% of patients with essential hypertension, treated and untreated, appear to be insulin resistant. And cardiovascular disease risk factors in these patients are greatly accentuated (Lima et al. 2009). Hyperlipidemia and hypertension are both included in the diagnostic criteria of MS, so hypertriglyceridemia patients with concurrent hypertension are at more risk to develop MS.

Peroxisome proliferator-activated receptor alpha (PPAR α) regulates genes involved in the metabolism of lipids, glucose, and amino acids (Peters et al. 2005). Links between PPAR α and the metabolic diseases including diabetes, obesity, and dyslipidemia have been suggested. This raises an untested hypothesis that PPAR α activation may be preventive for the progression of metabolic diseases. In addition to the studies on the treatment of hyperlipidemia, the differential effects of clinical PPAR α agonists on glucose metabolism were reported (Avogaro et al. 1995; Liu et al. 2011; Noguchi et al. 2011; Sheu et al. 1993). In a mouse model, sodium excretion was faster in *Ppara*-null mice than in wild-type mice (4.31 ± 1.11 vs. 0.77 ± 0.31 μ mol) in response to an acute NaCl load. This effect became less in amiloride-treated mice (3.4- vs. 15.5-fold) (Obih and Oyekan 2008). However, no reports were found about regulation of urine output by clinical PPAR α agonists directly.

Fibrate drugs are PPAR α agonists widely used for the treatment of hypertriglyceridemia and mixed hyperlipidemia associated with atherosclerosis (Fiévet and Staels 2009). They promote TG and very-low-density lipoprotein (VLDL) catabolism, which reduces the risk of cardiovascular events. However, gemfibrozil-mediated improvement in cardiovascular disease risk might not be the result of its effects on HDL-C. The cardiovascular benefits of gemfibrozil were supposed to be due to the effects on metabolic processes not reflected by its modification of lipid profiles (Asztalos et al. 2008). The role of fibrates in control of blood pressure, in addition to the classic pharmacodynamic effects on TG metabolism, is of great interest.

The effect of fibrate drugs on blood pressure has been reported. In Sprague Dawley rats receiving Ang II infusion, fenofibrate significantly reduced the systolic blood pressure, suggesting a role in the control of hypertension (Diep et al. 2004). A combination of fenofibrate and rosiglitazone in a rat model was reported to lead to attenuation of hypertension (De Ciuiceis et al. 2007). In type II diabetic subjects with left ventricular diastolic dysfunction, fenofibrate and coenzyme Q10 interactively lowered 24 h systolic blood pressure (Chew et al. 2008). In another study using a rat model, chronic treatment with the PPAR α agonist clofibrate treatment reduced salt-dependent arterial pressure (Williams et al. 2005). For bezafibrate, a significant reduction of systolic blood pressure and diastolic blood pressure was reported in patients with hypertension and hyperlipidemia (Kim et al. 2003). Hypertension is one of the most important markers in the clinic to diagnose MS cases. Diuretics belong to the first line agents for treatment of hypertension in the clinic and are the basic agents for combination with the other drugs. Thus, regulation of urine output is important for both patients with hypertension and those of MS. However, an association between attenuation of hypertension and urine output regulation by fibrates has not been suggested.

In this study, wild-type (WT) and *Ppara*-null mice were administered gemfibrozil and fenofibrate to evaluate the effects on urine output and potential kidney toxicity. Gemfibrozil was found to increase urine output with less toxicity compared with fenofibrate, which was dependent on PPAR α . These data suggest PPAR α 's role in regulating urine output and the potential use of gemfibrozil rather than fenofibrate to treat hypertriglyceridemic-hypertensive patients.

Materials and methods

Chemicals and reagents

Gemfibrozil was obtained from Hunan Qianjin Xiangjiang Pharmaceutical Co. Ltd. (Zhuzhou, China). Fenofibrate was purchased from Shangqiu Chemry Chemicals Co. Ltd. (Shangqiu, China). All the ELISA kits were purchased from B&D Systems (Shanghai, China). The reverse transcription kit was provided by Thermo Fisher (Pennsylvania, USA). The LightCycle 480 SYBR Green I Master Mix was purchased from Roche. Purified water was freshly prepared using a Millipore Elix (Massachusetts, USA) system. All the other chemicals were of the highest grade available from commercial sources.

Animals and treatment

WT and *Ppara*-null mice on the 129/Sv background, described previously (Lee et al. 1995; Yang et al. 2008), were gifted by Frank J. Gonzalez at National Cancer Institute, National Institutes of Health, USA. All mice were maintained in the animal center of Medical School of Ningbo University, under a standard 12 h light – 12 h dark cycle with free access to water and a commercial diet. All procedures were performed in accordance with Laboratory Animal Resource Guidelines (SYXK 2013–0191) and protocols approved by the Medical School of Ningbo University. Male 5- to 7-week-old mice were acclimated to a commercial purified diet for 7 days before the experiment. WT mice were fed a gemfibrozil (0.375%, 0.75%, and 1.5%) and fenofibrate (0.075%, 0.15%, and 0.3%) diet (WT-L, WT-M, and WT-H) respectively for 14 days where the dosage was designed based on their relative doses in the clinic. Considering the renal function response and urine volumes observed in the WT mice, the *Ppara*-null mice were fed high gemfibrozil and fenofibrate diets only (KO-H). The control groups for both mouse lines and agents were treated with purified diet only (WT-C and KO-C). All the mice were placed into metabowls on day 0, 7, and 14 during the treatment. Each mouse was weighed and food intake measured over 24 h. The urine samples over 24 h were collected using metabowls. The volumes were normalized by body mass and expressed as mL/10 g. Just before euthanasia by cervical dislocation, blood (as much as possible) was taken by retro-orbital bleeding for toxicity assay under CO₂ narcosis.

Because of the strong regulation, a sub-chronic experiment was performed for gemfibrozil, to find potential toxic endpoints and the reversibility of urine output regulation. Specifically, 5 WT and 5 *Ppara*-null mice were fed a high gemfibrozil diet for 2 months followed by a 2-week clearance. Urine output was measured on day 0, 30, and 60 after dosing ($n = 5$). Two mice from each group were killed on day 60. Urine output of the remaining 3 mice in both groups was measured 7 and 14 days after diet replacement, following which the mice were killed.

At the end of each experiment, all mice were weighed and killed after blood collection. Renal and liver tissues were harvested and weighed to calculate organ-to-body mass ratio. A section of renal and liver tissues were excised and immediately fixed in 10% neutral buffered formalin. The remaining tissues were flash frozen in liquid nitrogen. All urine, serum, and tissue samples were stored at –80 °C pending analysis.

Analysis of urine indicators

Glucose, hematuria, protein, bilirubin, urobilinogen, and ketone body in the urine were measured by clinical standard methods, to assay the potential toxic effect of urine output regulation.

Biochemical assessment of serum samples

The classic hepatotoxicity indicators alanine transaminase (ALT), aspartate transaminase (AST), creatinine, and blood urea nitrogen (BUN) were assayed by Spectra Max M5 (California, USA), using an end-point method or a dynamic method. Antidiuretic hormone (ADH) and aldosterone (ALD) levels were determined using ELISA kits (Shanghai, China). In the above determination, 2 μ L sample was added for each measurement. An aliquot of 50 μ L of the remaining sample was diluted (1:10) for measurement of K^+ , Na^+ , Ca^{2+} , and Cl^- by standard methods in the Affiliated Hospital of Medical College of Ningbo University.

Histopathological analysis

Fixed kidney tissues were subject to dehydration in a serial concentration of alcohol and xylene for paraffin embedding. Serial sections (4 μ m thick) were cut and stained with hematoxylin and eosin, followed by histological examination under an Olympus BX41 light microscope.

Quantitative polymerase chain reaction (Q-PCR) analysis

Expression of mRNAs encoding kidney transporters involved in the regulation of urine output, including aquaporins (AQP), sodium-chlorine cotransporter (NCC), sodium-hydrogen exchanger (NHE), and sodium-potassium-chloride cotransporter (NKCC) were measured. Total mRNA was extracted from approximately 20 mg frozen kidney tissues, using TRIzol reagent (Carlsbad, California, USA). Total RNA levels were determined by use of Nano Drop 2000 (Roche, USA). Reverse transcription was performed using standard procedures where first-strand cDNA was generated from 1 μ g total mRNA with a SuperScript II Reverse Transcriptase kit. The primers extracted from a public database (<http://mouseprimerdepot.nci.nih.gov/>) were tested for specificity and used in the PCR amplification. Each 10 μ L PCR system contained 1 μ L total cDNA, 5 μ L LightCycle 480 SYBR Green I Master Mix (FastStart Taq DNA Polymerase, reaction buffer, dNTP mix, SYBR Green I dye, and $MgCl_2$), 0.2 μ L forward primer, 0.2 μ L reverse primer, and 3.6 μ L nucleasefree water. The fluorescence signals were detected at the end of each cycle. Specific mRNA levels were normalized to 18S rRNA and expressed as fold change equivalent to that of WT-C.

Statistics

All data were expressed as the mean $\pm$ SD. SPSS 13.0 for Windows (Chicago, Illinois, USA) was used for the data analysis. The results following treatment in both WT and *Ppara*-null mice were compared with their baseline levels or those in their control groups respectively. A two-tailed, independent-sample *t* test was used to analyze the results from *Ppara*-null mice, and the organ indices or other indicators between WT mice and *Ppara*-null mice. One-way ANOVA followed by Fisher's least significant difference post hoc tests was used for

multiple comparisons of data from WT mice and those time-related alterations. A difference was considered significant when the p values were less than 0.05.

Results

Urine output regulated by gemfibrozil and fenofibrate

Compared with the WT-C group, WT mice dosed with low, medium, and high gemfibrozil exhibited increased urine output, with the volume of the urine output of the WT-H group about 6 times of that of WT-C group (Fig. 1A, $p < 0.05$). Compared with the WT-C group, no difference was found in KO-C group (Figs. 1A and 1B). No significant difference was found between the KO-C mice and KO-H mice (Fig. 1B, $p > 0.05$), indicating a critical role for PPAR α in regulation of urine output.

With fenofibrate, the urine output of WT-H mice doubled in comparison with that of the WT-C mice (Fig. 1C, $p < 0.05$). This increase was much smaller than that of gemfibrozil (2 times vs. 6 times). Similar to the gemfibrozil, there was no increase in urine output in the KO-H mice compared with the KO-C mice (Fig. 1D, $p < 0.05$). These results further demonstrated a role of PPAR in the regulation of urine output, with gemfibrozil more effective than fenofibrate in increasing urine output in the mouse model.

Toxic responses of liver and kidney

The liver-to-body mass ratios increased significantly in the 3 groups treated with gemfibrozil and fenofibrate compared with the WT-C group. However, it was evident that the liver-to-body mass ratio of the WT-H mice treated with gemfibrozil diet ($7.2 \pm 0.4\%$) was much smaller than those treated with fenofibrate ($10.1 \pm 0.9\%$) (Figs. 2A and 2B, $p < 0.05$). The liver-to-body mass ratio of the KO mice treated with high gemfibrozil and fenofibrate diets were significantly different compared with their control groups (Figs. 2C and 2D, $p > 0.05$). Biochemically, no changes in AST or ALT were found with either fibrate treatment (Supplementary Figures S1A–S1D¹), which correlated with the negative pathological result in the liver tissues (data not shown).

For the mass of kidney tissues, no difference was noted between control mice and treated mice (either WT or Ppara-null) for either fibrate (Figs. 2A–2D). In the clinic, increase of BUN and creatinine often suggest nephropathy. In the 14-day experiment, no increase in creatinine was found among those WT and KO groups. However, a significant difference of BUN was detected between WT-H and WT-C (Figs. 3A and 3B, $p < 0.05$). No histopathological changes in kidney tissues were observed in either mouse line after treatment with the gemfibrozil diets (Figs. 3C–3H). For the fenofibrate experiment, the kidney responses were similar as those of gemfibrozil (Supplementary Figure S2¹). The urine toxicity indicators glucose, hematuria, protein, bilirubin, urobilinogen, and ketone body were analyzed by clinical method after gemfibrozil and fenofibrate treatments, but none were changed, indicating no toxicity was associated with increased urine production (data not shown). For the 2 fibrate treatments, the biochemistry, pathology, and urine

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjpp-2016-0140>.

analysis results correlated well and indicated a tolerance for gemfibrozil and fenofibrate in the mouse kidney.

Serum homeostasis and mechanism exploration

No differences were observed for Na^+ , K^+ , Ca^{2+} , and Cl^- , between the WT and (or) *Ppara*-null mice after gemfibrozil treatment (Figs. 4A–4D). Difference was significant in *Ppara*-null mice for Na^+ and Cl^- except for K^+ . Additionally, modification in WT mice did not correlate with either the urine output or the serum ADH and ALD (Figs. 5A and 5B). Similar negative responses were found with fenofibrate treatment (Supplementary Figure S3¹).

AQPs are a group of water channels involving water transportation. *Aqp 1–3* and the ions transporter *Nhe*, *Ncc*, and *Nkcc* mRNAs known to be correlated with urine output were measured. However, no differences were found between the WT mice treated with gemfibrozil and the control group. Although some were down-regulated in *Ppara*-null mice, the decrease were less than 2-fold, thus indicating no biological significance (Figs. 5C and 5D). These data indicated that the mechanism of gemfibrozil alteration of urine output might not involve modification of transporter expression in the kidney.

Pharmacodynamic and toxic analysis of 2-month treatment

In the 2-month experiment, an increase of urine output was observed in WT mice treated with the high gemfibrozil diet. The maximal change was about 6 times of the baseline level, which was similar to the 14-day treatment and remained similar through 60 days ($n = 5$). Seven days after the diet replacement, the urine output quickly decreased and was almost normalized after another week ($n = 3$). As expected, no modification of urine output was observed during the 2-month drug challenge in the *Ppara*-null mice (Fig. 6A).

Phenotypically, the body mass of WT mice and *Ppara*-null mice did not change over the 2-month treatment (Fig. 6B). Creatinine was increased and went back to baseline upon drug withdrawal (Figs. 6C and 6D), and no morphological changes were found in the kidney tissues (Figs. 6E–6H). These data indicated kidney tolerance in the 2-month treatment with gemfibrozil.

Discussion

Clinically, reduction of blood pressure was reported to be associated with fibrate administrations (Borghetti et al. 2004; Kim et al. 2003; Williams et al. 2005). But the underlying mechanism has not yet been revealed. In a rat model, gemfibrozil was found to relax contraction of the tail artery and abolish the contractile activities of uterus, duodenum, and bladder (Phelps and Peuler 2010). A recent study revealed relaxation of rat thoracic aorta by gemfibrozil when gemfibrozil, bezafibrate, and fenofibric acid were compared. This effect was supposed to be associated with a reduction of intracellular calcium (Liu et al. 2012). The above suggested a reasonable pharmacodynamic basis for the reduction of blood pressure. In the present study, both gemfibrozil and fenofibrate were found to increase urine output in WT mice not in *Ppara*-null mice, suggesting a role for PPAR α in this new pharmacodynamic effect which might contribute to reduction of blood pressure.

The differential effect on MS by fibrate agents has been reported. In hypertriglyceridemic patients with and without diabetes mellitus in 2 different studies, gemfibrozil treatment improved glucose metabolism and insulin action ($n = 12$ and 11 , respectively) (Avogaro et al. 1995; Shen et al. 1991). Recently, gemfibrozil not fenofibrate was found to down-regulate systemic glucose level and glycogen storage in the liver dependent on PPAR α in a mouse model (Song et al. 2016). In a rat MS model, fenofibrate reduced the plasma lipid levels within a few days, but the fasting plasma insulin and insulin secretion response to glucose stimulation were also decreased (Liu et al. 2011). Additionally, no improvement of glucose tolerance and metabolic markers was observed with fenofibrate in dyslipidemia subjects with impaired glucose tolerance or type 2 diabetes mellitus (Noguchi et al. 2011). In this study, the urine output by gemfibrozil treatment was almost 6 times baseline in the WT mice, an effect stronger than that of fenofibrate (6-fold vs. 2-fold). Because high glucose level is often concurrent with hypertension in MS patients, the data in this study suggested stronger effectiveness of gemfibrozil for MS treatment compared with fenofibrate.

The therapeutic regimen of gemfibrozil in the clinic is about 1200 mg/day, with fenofibrate usually prescribed at 200–300 mg/day. In this study, “low”, “medium”, and “high” gemfibrozil doses (0.375%, 0.75%, and 1.5%, respectively) and fenofibrate doses (0.075%, 0.15%, and 0.3%) were administered in pelleted diets for 14 days. Compared with the 69% increase of liver-to-body mass ratio noted with high-gemfibrozil treatment, the 120% increase in the high-fenofibrate group indicated a higher risk of hepatomegaly for fenofibrate. This difference should be taken into account for investigations in rodent models, because complicated biochemical events occur underlying hepatomegaly. However, it is not a big concern in the clinical situation because human livers are refractory to this pharmacodynamic effect of PPAR α agonists.

A reversible rise of serum creatinine associated with fibrates was reported in the clinic. Increases of creatinine production and a reduction of prostaglandins have been suggested as the underlying mechanism (Sica 2009). In another a retrospective clinical report, 10 male patients showed a reversible deterioration in serum creatinine when treated with a fibrate agent (pre-treatment: 182 ± 14 $\mu\text{mol/L}$, medication: 247 ± 16 $\mu\text{mol/L}$, post-medication: 183 ± 13 $\mu\text{mol/L}$). The mechanism involved was related to renal hemodynamics (Lipscombe et al. 2001). In this study, kidney related toxicity was systemically assayed. With a 14-day treatment of either gemfibrozil or fenofibrate, no modification of serum biochemical markers or histopathology was observed. In the 2-month treatment with gemfibrozil, the toxic responses were similar as those of the 14-day treatment except that the increase of serum creatinine in WT mice was observed on day 60 which decreased to the levels found in *Ppara*-null mice.

Diuretics are recommended as first-line drugs in early treatment of patients with hypertension in the clinic. However, most of the diuretics lead to high risk of electrolyte disturbances and endocrine disorders (Chobanian et al. 2003). In this study, 3 ions were modified between the KO-C and KO-H after gemfibrozil treatment. Na⁺ and Cl[−] changed very mildly except for K⁺ in KO mice. None of them were altered by fenofibrate in either mouse line (Supplementary Figure S3¹). Additionally, a change was noted in ADH and ALD among the groups of WT and *Ppara*-null mice in this study. AQP3, which plays a crucial

role in glycerol metabolism dependent on PPAR (Patsouris et al. 2004), was not modified in kidney tissues in either WT or *Ppara*-null mice in this study. However, transcription of kidney transporters is only one of the steps in urine output regulation. Therefore, it remains to be investigated whether there is a mechanism related with transporters. Considering the disagreement of the above biochemistry with pathology and phenotype, it was more reasonable to attribute these variations to metabolic regulation instead of diuresis.

In conclusion, a regulatory effect on urine output mediated by PPAR α was revealed with clinically prescribed PPAR α agonists and transgenic mice. Gemfibrozil increased urine output with less risk of hepatomegaly compared with fenofibrate. These data revealed a differential potential between gemfibrozil and fenofibrate for the treatment of hypertriglyceridemia patients with concurrent hypertension. However, the exact mechanism underlying increase of urine output by gemfibrozil remains to be investigated. Considering the species difference related with PPAR α , these data in mice may not reflect results in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

The urine output regulated in wild-type (WT) and *Ppara*-null (KO) mice treated with gemfibrozil or fenofibrate. (A) The urine output in the WT mice treated with gemfibrozil for 14 days. (B) The urine output in the KO mice treated with gemfibrozil for 14 days. (C) Urine output of WT mice treated with fenofibrate for 14 days. (D) Urine output of KO mice treated with fenofibrate. Data were expressed as mean $\pm$ SD ($n = 5$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant).

Fig. 2.

Organ indices in wild-type (WT) and *Ppara*-null (KO) mice treated with gemfibrozil or fenofibrate. (A) Organ indices in the WT mice treated with gemfibrozil. (B) Organ indices in the WT mice treated with fenofibrate. (C) Organ indices in KO mice treated with gemfibrozil. (D) Organ indices in the KO mice treated with fenofibrate. Data were expressed as mean $\pm$ SD ($n = 5$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant).

Fig. 3.

Biochemical and pathological responses of the wild-type (WT) and *Ppara* α -null (KO) mice treated with gemfibrozil for 14 days. (A) Serum levels of creatinine (CRE). (B) Serum levels of blood urine nitrogen (BUN). (C–H) Representative H&E staining of kidneys from WT-C, WT-L, WT-M, WT-H, KO-C, and KO-H mice. Mice were fed with control-, low-, medium-, high-gemfibrozil diets for 14 days. CRE and BUN were measured by clinical detection methods. Data were expressed as mean $\pm$ SD ($n = 5$; ** $p < 0.01$; ns, not significant). [Colour online.]

Fig. 4.

Serum ion levels in mice treated with different doses of gemfibrozil. (A) Serum Na⁺. (B) Serum K⁺. (C) Serum Ca²⁺. (D) Serum Cl⁻. Mice were fed with control-, low-, medium- and high-gemfibrozil diets for 14 days. Data were expressed as mean $\pm$ SD ($n = 5$; * $p < 0.05$; ns, not significant; WT, wild-type; KO, *Ppara*-null).

Fig. 5.

Hormone and mRNA levels related to urine production in mice treated with gemfibrozil. (A) Serum antidiuretic hormone (ADH). (B) Serum aldosterone (ALD). (C) *Aqp1*, *Aqp2*, *Aqp3* mRNAs. (D) *Nhe*, *Ncc*, *Nkcc1*, *Nkcc2* mRNAs. Serum hormones levels were measured by ELISA kit. Data were from kidney samples and the mRNA levels were measured by qPCR and normalized by 18S rRNA. mRNA levels in WT-C mice were arbitrarily set as 1 and data were expressed as mean $\pm$ SD ($n = 5$; * $p < 0.05$; *** $p < 0.001$; ns, not significant; WT, wild-type; KO, *Ppara*-null).

Fig. 6.

The phenotype, biochemical, and pathological responses in the 2-month experiment of gemfibrozil. (A) The urine output of mice treated with high-gemfibrozil for 2 months. (B) Body masses of mice treated with high-gemfibrozil for 2 months. (C) Serum creatinine (CRE) in the 2-month gemfibrozil experiment. (D) Serum blood urea nitrogen (BUN) in 2-month gemfibrozil experiment. (E–H) Representative pictures of histological analysis of the kidney in mice treated with gemfibrozil. H&E staining of kidneys from WT-2m, KO-2m, WT-2m + 14d, KO-2m + 14d mice (WT, wild-type; KO, *Ppara*-null; d, days after dosing; c, days after clearance). [Colour online.]