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Peroxisome proliferator-activated receptor α -dependent renoprotection of murine kidney by irbesartan

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Abstract

Activation of renal peroxisome proliferator-activated receptor α (PPAR α) is renoprotective, but there is no safe PPAR α activator for patients with chronic kidney disease (CKD). Studies have reported that irbesartan (Irbe), an angiotensin II receptor blocker (ARB) widely prescribed for CKD, activates hepatic PPAR α . However, Irbe's renal PPAR α -activating effects and the role of PPAR α signalling in the renoprotective effects of Irbe are unknown. Herein, these aspects were investigated in healthy kidneys of wild-type (WT) and *Ppara*-null (KO) mice and in the murine protein-overload nephropathy (PON) model respectively. The results were compared with those of losartan (Los), another ARB that does not activate PPAR α . PPAR α and its target gene expression were significantly increased only in the kidneys of Irbe-treated WT mice and not in KO or Los-treated mice, suggesting that the renal PPAR α -activating effect was Irbe-specific. Irbe-treated-PON-WT mice exhibited decreased urine protein excretion, tubular injury, oxidative stress (OS), and pro-inflammatory and apoptosis-stimulating responses, and they exhibited maintenance of fatty acid metabolism. Furthermore, the expression of PPAR α and that of its target mRNAs encoding proteins involved in OS, pro-inflammatory responses, apoptosis and fatty acid metabolism was maintained upon Irbe treatment. These renoprotective effects of Irbe were reversed by the PPAR α antagonist MK886 and were not detected in Irbe-treated-PON-KO mice. These results suggest that Irbe activates renal PPAR α and that the resultant increased PPAR α signalling mediates its renoprotective effects.

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AUTHOR CONTRIBUTION

Makoto Harada, Yuji Kamijo, Frank Gonzalez and Toshifumi Aoyama designed the study. Makoto Harada, Takero Nakajima, Koji Hashimoto and Yosuke Yamada performed experiments and acquired the data. Makoto Harada and Hisashi Shimojo analysed pathological findings and acquired the data. Makoto Harada and Yuji Kamijo analysed the data and drafted the manuscript.

Keywords

angiotensin II type 1 receptor blocker; fatty acid metabolism; irbesartan (Irbe); peroxisome proliferator-activated receptor α (PPAR α)

INTRODUCTION

Peroxisome proliferator-activated receptor α (PPAR α) is a member of the steroid/nuclear receptor superfamily and is distributed to fatty acid-consuming organs including the kidney and liver [1,2]. PPAR α activation increases the expression of its target genes encoding enzymes involved in fatty acid transport and metabolism. In kidney, PPAR α is mainly expressed in the proximal tubular epithelial cells (PTECs), and maintains energy homeostasis and tubular absorptive function [3]. Kidney injury decreases renal PPAR α expression, and PPAR α agonist-induced renal PPAR α activation exerted tubular-protective effects in murine models [4,5]. Furthermore, renal PPAR α activation might suppress the progression of renal fibrosis and the decline of kidney function in chronic kidney disease (CKD) [6].

Among the known PPAR α agonists, fibrates are used clinically in the treatment of hyperlipidaemia [7]. Fenofibrate could suppress microalbuminuria in patients with diabetes mellitus (DM) nephropathy, suggesting a potential decrease in the glomerular filtration rate loss [8–10]. However, the serum concentration of fibrates in CKD patients is markedly increased and is frequently followed by renal toxicity [10]. Currently, safe clinical methods for activating renal PPAR α in CKD patients have not been established.

Two angiotensin II receptor blockers (ARBs), telmisartan (Tel) and irbesartan (Irbe) are well known for their partial agonist activity for PPAR γ [11–15]. In addition, Tel and Irbe can also activate hepatic PPAR α [16,17]. However, whether they activate renal PPAR α is not well understood. Irbe shows high renal distribution; therefore, Irbe might activate renal PPAR α [18]. Irbe is widely used in CKD patients as a valuable antihypertensive medicine by suppressing the renin–angiotensin–aldosterone system (RAS). Several randomized control trials using Irbe demonstrated significant renoprotection independent of Irbe's antihypertensive effects in DM patients [19,20]. Renal PPAR α activation by Irbe may contribute to these effects, but Irbe's renal PPAR α -activating effects and the role of PPAR α signalling in the renoprotective effects of Irbe are unknown.

The present study shows that Irbe treatment could activate renal PPAR α and exerted renoprotective effects via renal PPAR α activation in the murine protein-overload nephropathy (PON) model, induced by the intraperitoneal administration of a large amount of free (non-esterified) fatty acid-conjugated BSA (FFABSA) [21]. PON mice exhibit severe proteinuria without glomerular injury, and exhibit acute tubular injury due to excessive FFA-BSA reabsorption in PTECs [21]. The mechanism underlying kidney injury in PON is closely related to FFA overload rather than to BSA overload. Intracellularly accumulated FFAs exert lipotoxicity (LTx) and cause FFA metabolic dysfunction associated with PPAR α loss, inflammation, increased oxidative stress (OS), apoptosis stimulation and severe PTEC injury [4,21]. PPAR α deficiency dramatically aggravated LTx and tubular damage in PON,

which were reduced by the renal PPAR α -activating effect of the PPAR α agonist clofibrate [4,22], suggesting that PON is a suitable animal model for analysing the renoprotective effects of PPAR α activation. Therefore, PON herein, was used for the evaluation of Irbe's novel pharmacological renal PPAR α activation effects. The most important beneficial effects of renal PPAR α activation are increased fatty acid metabolism, which reduces LTx derived from proteinuria. To clarify the renoprotective effects mediated by PPAR α in suppressing the initial step of kidney injury in PON, we employed a pretreatment protocol in which Irbe treatment was started before a kidney injury episode.

MATERIALS AND METHODS

Animals and experimental design

Male wild-type (WT) mice and *Ppara*-null (KO) mice (age, 13 weeks; body weight, 24–30 g) were used in the present study. WT and KO mice were on a C57BL6 background, as previously described [23]. All procedures were performed in accordance with the guidelines of Shinshu University, the National Institutes of Health and the Association for Assessment and Accreditation of Laboratory Animal Care.

Study 1—WT and KO mice were divided into three groups: a control group (vehicle- [0.1% methylcellulose, MC] treated group, $n = 10$ and $n = 6$ respectively), Los-treated group ($n = 10$ and $n = 6$ respectively) and Irbe-treated group ($n = 10$ and $n = 6$ respectively) (50 bw/day of each ARB for 14 days). The chemicals were administered via oral gavage. After 14 days, the mice were killed.

Study 2—WT and KO mice were administered a daily intra-peritoneal bolus injection of 0.3 g of FFA-BSA in sterile saline for 4 days, and the dose was increased (0.35 g for 3 days and 0.4 g for 7 days) to induce PON [21,22]. The following WT and KO mice groups were used: vehicle- (0.1% MC) treated mice without PON (Con, $n = 10$ and $n = 6$ respectively), vehicle-treated mice with PON (Veh-PON, $n = 6$ and $n = 6$ respectively), Los-treated mice with PON (Los-PON, $n = 6$ and $n = 6$ respectively), Irbe-treated mice with PON (Irbe-PON, $n = 6$ and $n = 6$ respectively), and Irbe and MK886 (PPAR α antagonist)-treated mice with PON (Irbe-MKPON, $n = 6$, WT only). The vehicle or respective ARB (50 mg/kg bw/day) and PPAR α antagonist (MK886, 1 mg/kg, intraperitoneally) were administered every day from Day 1 to Day 20. BSA was injected intraperitoneally every day from Day 7 to Day 20. All groups of WT mice were killed at Day 20. The procedures related to the preliminary experiments for the determination of the appropriate doses of Irbe and Los, as well as to the effect of MK886 on blood pressure, are described in the Supplementary Methods.

Details of methods for analyses

Details of analysis methods and miscellaneous methods are described in Supplementary Methods and Supplementary Table S1 [24].

Statistical analyses

Significant differences with respect to the interactive effects were analysed using one-way ANOVA, and Tukey test for multiple comparisons, correspondingly. Values represent the

mean $\pm$ S.D. Probability values less than 0.05 were considered significantly different. Statistical analyses were performed by using the SPSS Statistics version 22.0J (IBM), and Stat Flex version.6 for Windows (ARTECH).

RESULTS

Irbe activates PPAR α in kidney tissues

Vehicle, Irbe and losartan (Los; an ARB without PPAR α agonist activity) were administered to WT and *Ppara*-null (KO) mice, and their effects were compared. The expression of *Ppara* mRNA, PPAR α protein, PPAR response element (PPRE) binding of intra-nuclear PPAR α , and the mRNA expression and protein amount of the representative PPAR α target genes encoding long-chain acyl-CoA synthetase (LACS) and peroxisomal thiolase (PT) were significantly increased in the kidneys of Irbe-treated WT mice (Figure 1). No changes in the expression of renal PPAR α and its target genes were detected in the kidneys of Los-treated WT mice and in those of all KO mice groups.

Since Irbe activates PPAR γ , another PPAR subtype, PPAR γ expression was also determined, and no changes in *Pparg* mRNA and PPRE binding ability of PPAR γ and a representative PPAR γ target gene, adipocyte fatty acid binding protein 2 (AP2), were detected in the kidneys of all mice groups.

Irbe treatment exerts renoprotective effects in PON-WT mice

The renoprotective effects of Irbe were evaluated in the PON model using WT mice. None of the mice died during the experimental period. The systemic alterations were compared among the five treatment groups (Figure 2). The systolic blood pressure in the Veh-PON group increased from Day 16 (Figure 2A), whereas the systolic blood pressure in all ARB-PON groups rapidly decreased after administration of the respective ARB. These hypotensive effects were observed during the entire experimental period, and were identical among the groups. The urine volumes were significantly and identically increased from Day 16 in all PON groups (Figure 2B). The urine protein excretion and urine *N*-acetyl- β -D-glucosaminidase (NAG)/urine creatinine (Cr) ratio were greatly and identically increased in the Veh-PON, Los-PON and Irbe-MK-PON groups. On the other hand, those in the Irbe-PON group were significantly attenuated and were maintained at levels similar to those of the Con group (Figures 2C and 2D). Kidney sections revealed extensive swelling of the renal cortices in the Veh-PON, Los-PON and Irbe-MK-PON groups, whereas the renal cortices in the Irbe-PON group appeared normal (Figure 3A). Light microscopic analysis showed tubular injury consisting of tubular dilation and sloughing of PTECs in the Veh-PON, Los-PON and Irbe-MK-PON groups, whereas these injuries in the Irbe-PON group were markedly attenuated (Figure 3B). Immunohistochemical analysis showed strong positive staining for osteopontin, a pathological marker of tubular injury, in PTECs in the Veh-PON, Los-PON and Irbe-MK-PON groups, whereas only slightly positive staining was observed in the Irbe-PON group (Figure 3C). Electron microscopic analysis revealed morphological changes of the mitochondria, i.e. swelling and rupture, in PTECs in the Veh-PON, Los-PON and Irbe-MK-PON groups; such changes were scarcely detected in the Irbe-PON group (Figure 3D). A semi-quantitative scoring analysis demonstrated that the severity of tubular

injury in the Irbe-PON group was significantly attenuated (Figure 3E). Pathological analyses also revealed glomerular swelling in all of the PON groups; however, other glomerular lesions including podocyte damage were not detected in all of the PON groups (Supplementary Figure S1).

The renoprotective effects of Irbe are associated with improvements in OS, inflammation, apoptosis and FFA metabolism

The level of blood urea nitrogen (BUN) was significantly increased in the Irbe-PON group; however, the serum Cr levels did not differ among the groups (Figure 4A). PON-induced tubular injuries are related to intracellular LTx causing OS, inflammation and apoptosis; therefore, these factors were compared among the five groups. Immunoblot analysis and a colorimetric assay revealed that markers for tissue OS, i.e. renal 4-hydroxy-2-nonenal-(4-HNE) modified proteins and oxidized lipids (malondialdehyde [MDA] + 4-HNE) were significantly and identically increased in the Veh-PON, Los-PON and Irbe-MK-PON groups, whereas they were attenuated in the Irbe-PON group (Figure 4B). The expression of mRNA encoding the reactive oxygen species-generating oxidative enzymes nicotinamide adenine dinucleotide phosphate oxidase 4 (Nox4) and acyl-CoA oxidase (ACOX), and that of the antioxidant enzymes catalase (Cat) and Cu, Zn-superoxide dismutase (SOD1) was examined. The expression of *Nox4* and *Sod1* mRNA did not differ among the five groups. Expression of mRNA for *Acox* and *Cat*, which are representative PPAR α -regulated molecules, was significantly decreased in the Veh-PON, Los-PON and Irbe-MK-PON groups. In the Irbe-PON group, *Acox* mRNA levels were similar to that of the Con group, whereas that of *Cat* mRNA was significantly increased compared with that of the Con group.

The expression of mRNA encoding pro-inflammatory mediators, tumor necrosis factor α (TNF α), cyclooxygenase 2 (COX2) and intercellular adhesion molecule 1 (ICAM1) was significantly and identically increased, and the mRNA expression of I κ B α , an important PPAR α -regulated anti-inflammatory molecule, was decreased in the Veh-PON, Los-PON and Irbe-MK-PON groups (Figure 5A). In contrast, the expression of mRNAs encoding respective proteins in the Irbe-PON group was similar to that in the Con group.

The expression of *Bclx1* and *Bcl2* mRNA transcripts, which encode two anti-apoptotic, PPAR α -regulated molecules controlling mitochondrial permeability, was significantly decreased in the Veh-PON, Los-PON and Irbe-MK-PON groups, whereas *Bclx1* and *Bcl2* expression in the Irbe-PON group was identical with that in the Con group (Figure 5B). The expression of *Bax* and *Bid* mRNAs, encoding two apoptosis-stimulating molecules, did not differ among the five groups.

To assess LTx, changes in the renal FFA levels were analysed. The FFA serum levels were dramatically increased by PON, but did not differ among the PON groups, suggesting an identical renal FFA overload in all PON groups (Figure 5C). The renal tissue FFA content was greatly increased, and the expression of mRNA encoding LACS and carnitine palmitoyltransferase 1 α (CPT1 α), which are two important PPAR α -regulated fatty acid metabolic enzymes, was significantly decreased in the Veh-PON, Los-PON and Irbe-MK-PON groups. However, the mRNA expression of LACS and CPT1 α in the Irbe-PON group was similar to those in the Con group.

The mRNA expression, amount of intra-nuclear protein and PPRE binding ability of PPAR α were significantly decreased in the kidney tissues of the Veh-PON, Los-PON and Irbe-MK-PON groups, but in the Irbe-PON group, they were maintained equal to or increased beyond the levels in the Con group (Figure 6). PPAR γ expression did not differ among the five groups.

Renoprotective effects of Irbe are not detected in PON-KO mice

We investigated the renoprotective effects of ARBs in PON-KO mice. Previous studies demonstrated that KO mice are highly sensitive to PON, and that PON-KO mice generally exhibit severe kidney injury and high mortality [4]. As previously reported, almost all PON-KO mice in the present study died, irrespective of ARB treatment for severe kidney injury, within 7 days. The mortality was identical among PON-KO groups. The representative pathological findings of PON-KO mice on Day 4 are shown in Figure 7. Severe tubular injury is observed in KO + Veh + PON, KO + Los + PON and KO + Irbe + PON mice. None of the renoprotective effects of Irbe were detected in PON-KO mice.

DISCUSSION

The current study revealed that Irbe treatment increased expression of PPAR α and PPAR α signalling in kidney tissue. Because these changes were not detected in the kidneys of KO mice, these were most likely related to PPAR α activation. Los treatment did not activate PPAR α and thus activation of PPAR α by Irbe was Irbe-specific rather than due to the suppression of RAS, a common ARB class effect. Furthermore, the present results suggest that Irbe exerts its renoprotective effects through renal PPAR α activation, maintaining fatty acid metabolic homeostasis along with anti-oxidative, anti-inflammatory and anti-apoptotic effects as well as decreased tubular injuries. The reversion of Irbe-mediated renal protection by PPAR α antagonists and the insufficient renoprotective effect in KO mice strongly supports a PPAR α -mediated mechanism for the renoprotective effects of Irbe treatment. These effects were consistent with those found in previous studies using fibrates [22,25,26]. Since fibrates may exert nephrotoxicity that exceeds their renoprotective effects in kidney dysfunction, Irbe treatment may be a safer alternative [22].

Proteinuria causes tubulointerstitial injuries (TI) and is an important common risk factor for CKD progression [4,27,28]. Furthermore, renal prognosis is closely correlated to the degree of TI [29]. The mechanism underlying proteinuric toxicity is controversial, but several studies showed the importance of FFAs [4]. The large amount of FFAs bound to albumin, filtered through damaged glomeruli, is linked to increased absorption of FFAs by PTECs beyond metabolic capacity. As a result, the increase in un-metabolized intracellular FFAs causes LTx and TI [4]. A decrease in FFA metabolism associated with reduced PPAR α expression was one of the most important risk factors causing TI and CKD progression [6]. Therefore, drugs maintaining renal PPAR α levels, such as Irbe, may protect against TI and suppress the progression of CKD.

The current study suggests that Irbe treatment has anti-inflammatory, anti-oxidative and anti-apoptosis effects; these effects have previously been reported [30–37]. Experimental and clinical studies showed Irbe treatment-induced suppression of kidney inflammation, and

reduced serum levels of monocyte chemoattractant protein-1 (MCP-1), TNF α and interleukin-6 (IL-6) [30–32]. Irbe treatment increased the expression of anti-oxidative enzymes SOD1, glutathione peroxidase and Cat, and decreased serum oxidative markers, such as reactive oxygen species and MDA in a rat model and patients with hypertension or DM [33–35]. Moreover, Irbe treatment suppressed PTEC apoptosis caused by protein overload or contrast agents [36,37]. These pharmacological effects of Irbe may be associated with PPAR activation.

RAS activation causes hypertension and kidney injuries, and increases the expression of inflammatory mediators and OS [38–40]. Hypertension induces apoptosis in multiple organs including the kidneys [41]. RAS activation occurs in PON and aggravates proteinuria and TI, and RAS inhibition by ARB had renoprotective effects against PON [42,43]. In the current study, however, no significant attenuation of TI was observed in the Los-PON group. Since the BSA injection period was longer and the BSA dose was higher than those in previous studies, these severe experimental conditions might have concealed the protective effects of RAS inhibition. Nevertheless, Irbe treatment exerted renoprotective effects under these conditions. Several reports have indicated an interesting relationship between RAS and PPAR α : a decrease in renal PPAR α causes RAS activation, inflammation and OS, which are attenuated by PPAR α activation [44,45]. These findings suggest that PPAR α activation enhances the renoprotective effects of RAS inhibition. Therefore, Irbe treatment may attenuate the severe PON-induced TI by a dual effect of RAS inhibition and PPAR α activation.

The current study suggests that Irbe treatment exerts a strong anti-proteinuric effect against PON. In the PON model, a relatively large amount of overloaded BSA, i.e. exceeding the reabsorption ability of PTECs, is filtered from non-damaged glomeruli (Supplementary Figure S1). Since the mechanism underlying glomerular protein filtration in PON is only marginally dependent on the intraglomerular pressure, the glomerular protective effects and blood pressure-lowering effects of ARBs do not significantly affect the large amount of urine protein. Both the Veh-PON and Los-PON groups exhibited severe TI after BSA-induced LTx. Therefore, the BSA tubular reabsorption capacity was strongly impaired in these treatment groups, resulting in equally high proteinuric levels. On the other hand, tubular damage was only mildly present in the Irbe-PON group with preservation of the BSA tubular reabsorption capacity, which was reflected by decreased urinary protein levels in this treatment group.

Interestingly, levels of BUN were significantly higher in the Irbe-PON group. Because the increase in BUN levels was not related to the other kidney injury markers in the Irbe-PON group, we postulated that the increased BUN levels did not reflect kidney injury. We thus hypothesize that the mechanism underlying the increased BUN levels in the Irbe-PON group is as follows: Irbe activates renal PPAR α and increases FFA metabolic ability, resulting in the maintenance of energy homeostasis and reabsorption capacity of the PTECs. As a result, the reabsorption and catabolism of overloaded BSA were increased in the Irbe-PON group as detected by the increase in serum protein and BUN. Indeed, serum albumin and the total protein level were increased in Irbe-PON group (Supplementary Table S2), supporting our hypothesis. We considered that BUN is not a suitable marker for evaluating kidney

function in the PON model, and other kidney injury markers should be included in future studies using this model.

In the current study, Irbe treatment might have activated systemic PPAR α and PPAR γ , which are known to improve fatty acid metabolism, insulin tolerance and glucose metabolism [11–17]. It is possible that the resulting systemic metabolic improvement could have indirect renoprotective effects. In particular, these effects might decrease the degree of renal FFA overload in the Irbe-PON group, although significant reduction in the level of serum FFA was not detected in the current study. Furthermore, vascular PPAR α and PPAR γ have been shown to exert anti-inflammatory and anti-atherosclerotic effects [14,15,46,47], suggesting that Irbe treatment might protect against potential PON-induced vascular injuries.

Because renal PPAR γ activation has been shown to have renoprotective effects [11–15], it is important to consider the possibility of renal PPAR γ activation by Irbe treatment. In the current study, increased mRNA expression and PPRE binding ability of PPAR γ in the Irbe-treated WT group were not detected. Moreover, if Irbe had a significant renoprotective effect via PPAR γ activation, we would have detected this in the Irbe-MK-PON WT and Irbe-PON-KO group. However, we could not find any renoprotective effect in these treatment groups. From these results, we concluded that the renoprotective effects were mainly due to PPAR α activation rather than PPAR γ activation. However, we cannot entirely deny the possibility of undetected renoprotective effects of PPAR γ activation. In the present experiments using KO mice, the kidney injury was too severe to evaluate the protective effects of ARBs; therefore, the reno-protective effect of Irbe mediated by PPAR γ might have been masked by the severe kidney injury. In addition, given that the expression of PPAR γ is much smaller than that of PPAR α in kidney tissue, a potential renoprotective effect via PPAR γ activation may not be detected. Thus, various undetected effects of Irbe might have contributed to the renoprotective effects in the PON model.

There are several limitations in the present study. First, we used a tail cuff method to measure blood pressure. The tail cuff method generally is associated with wider blood pressure variation than continuous arterial blood pressure monitoring. Therefore, it is possible that we could not detect significant differences in blood pressure among the groups because of measurement variability. However, our preliminary experiments and previous studies demonstrated that the blood pressure-lowering effects of Los and Irbe are identical at the same milligram (mg) dosage (Supplementary Methods). One of the main mechanisms underlying the renoprotective effects of ARBs is the lowering of blood pressure; therefore, the renoprotective effect through blood pressure-lowering is identical between Irbe and Los treatment. On the other hand, the current study demonstrated that Irbe had a stronger renoprotective effect than observed for Los. We conclude that the additional renoprotective effect of Irbe beyond blood pressure-lowering is strongly associated with renal PPAR α activation.

Further, we employed a pretreatment protocol in the present study. The main mechanism underlying the renoprotective effect of Irbe is the activation of renal PPAR α and the increase in the metabolic capacity of FFA binding to urinary protein. Thus, the mechanism underlying the observed renoprotective effect is the elimination of important tubular toxic

factors, i.e. FFAs, thereby preventing overt kidney injury. Therefore, we postulate that the renoprotective effects of Irbe after the development of PON may be attenuated. It is still unknown whether or not PPAR α activation by Irbe treatment can improve renal morphology and function after PON is established; however, for the clinical applicability of Irbe treatment, it is important that Irbe successfully attenuates fatty acid toxicity, especially at the early stages of kidney disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ACOX	acyl-CoA oxidase
AP2	adipocyte fatty acid binding protein 2
ARB	angiotensin II receptor blocker
BUN	blood urea nitrogen
Cat	catalase
CKD	chronic kidney disease
COX2	cyclooxygenase 2
CPT1α	carnitine palmitoyltransferase 1 α
Cr	creatinine
DM	diabetes mellitus
FFA	free (non-esterified) fatty acid
4-HNE	4-hydroxy-2-nonenal
ICAM1	intercellular adhesion molecule 1
Irbe	irbesartan
Irbe-MK-PON	MK886-treated mice with PON
Irbe-PON	Irbe-treated mice with PON

LACS	long-chain acyl-CoA synthetase
Los	losartan
Los-PON	Los-treated mice with PON
LTx	lipotoxicity
MDA	malondialdehyde
NAG	<i>N</i> -acetyl- β -D-glucosaminidase
Nox4	nicotinamide adenine dinucleotide phosphate oxidase 4
OS	oxidative stress
PON	protein-overload nephropathy
PPARα	peroxisome proliferator-activated receptor α
PPARγ	peroxisome proliferator-activated receptor γ
PPRE	PPAR response element
PT	peroxisomal thiolase
PTEC	proximal tubular epithelial cell
RAS	renin–angiotensin–aldosterone system
SOD1	Cu, Zn-superoxide dismutase
Tel	telmisartan
TI	tubulointerstitial injuries
TNFα	tumor necrosis factor α
Veh-PON	vehicle-treated mice with PON
WT	wild-type

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CLINICAL PERSPECTIVES

- Activation of renal PPAR α is renoprotective, but there is no safe PPAR α activator for patients with CKD. Irbe may activate renal PPAR α ; however, Irbe's renal PPAR α -activating effects and the role of PPAR α signalling in the renoprotective effects of Irbe are unknown.
- The current study shows for the first time that Irbe treatment can activate renal PPAR α and exert PPAR α -dependent reno-protective effects in the murine PON model.
- These results suggest potential renoprotective clinical benefit of renal PPAR α activation by Irbe in human CKD patients with proteinuria. This safe clinical method for activating renal PPAR α may be a novel therapeutic strategy against CKD in future. Irbe may be a promising drug for patients with kidney disease.

Figure 1. PPAR α , PPAR γ and their target gene expression after each respective treatment in kidneys of WT and *Ppara*-null (KO) mice

(A) Expression of mRNAs encoding PPAR α , PPAR γ and their target genes LACS, PT and AP2. (B) PPAR α and its target gene protein (LACS and PT) levels. (C) PPARE binding ability of PPAR α and PPAR γ in each group. Con, control; Los, losartan; Irbe, irbesartan. Values represent the mean $\pm$ S.D. ($n = 10$ for WT; $n = 6$ for KO). We compared the data among treatment each genotype by using one-way ANOVA and Tukey's test. Significant differences are indicated with $\#P < 0.05$.

Figure 2. The study protocol of PON and the alterations in the systemic parameters in each group of WT mice with PON
 (A) Systolic blood pressure, (B) total urine volume, (C) total urine protein and (D) ratio of urine NAG/urine Cr in each group are indicated. MK, MK886. Significant differences compared with Day 0 in each group using Tukey's test, are indicated with * $P < 0.05$; significant differences compared with the Veh-PON group by using one-way ANOVA and Tukey's test are indicated with # $P < 0.05$. Values represent the mean $\pm$ S.D. ($n=10$ for Con; $n=6$ for treatment groups).

Figure 3. Pathological findings in each group of WT mice with PON

(A) Low-magnification histological photographs of kidney sections stained with haematoxylin and eosin. Scale bar 2 mm. (B) Light microscopy analyses of tubular lesions. Kidney sections were stained with periodic acid-methenamine-silver. Scale bar = 50 μm . (C) Immunohistochemical stains for osteopontin. Damaged PTECs were positively stained for osteopontin. Scale bar = 100 μm . (D) Electron micrographs of mitochondria in PTECs. Scale bar = 600 nm. (E) The results of the semi-quantitative tubular damaging score. The scores represent the mean $\pm$ S.D. ($n=10$ for Con; $n=6$ for treatment groups). Significant differences one-way ANOVA and Tukey's test are indicated with # $P < 0.05$.

Figure 4. The alterations in the renal function and OS markers in kidney tissues of each group of WT mice with PON

(A) Serum levels of BUN and serum Cr in each group of mice. (B) The alterations in the tissue OS markers (4-HNE-modified proteins), the amount of oxidative lipids (malondialdehyde + 4-HNE), and *Nox4*, *Acox*, *Cat* and *Sod1* mRNAs. Values represent the mean $\pm$ S.D. ($n = 10$ for Con; $n = 6$ for treatment groups). Significant differences by one-way ANOVA and Tukey's test are indicated with $\#P < 0.05$.

Figure 5. The alterations in the pro-inflammatory mediators, apoptosis-related molecules and FFA metabolism in the kidney tissues of each group of WT mice with PON
(A) *Tnfa*, *Cox2*, *Icam1* and *Ikba* mRNA levels. **(B)** *Bclxl*, *Bcl2*, *Bax* and *Bid* mRNA levels. **(C)** Serum and renal FFA content, and *Lacs* and *Cpt1a* mRNA levels. Values represent the mean $\pm$ S.D. ($n = 10$ for Con; $n = 6$ for treatment groups). Significant differences by one-way ANOVA and Tukey's test are indicated with # $P < 0.05$.

Figure 6. PPAR α and PPAR γ expression in kidney tissues of each group of WT mice with PON (A) *Ppara* and *Pparg* mRNA levels. (B) Intra-nuclear PPAR α protein expression. (C) PPRE binding ability of PPAR α and PPAR γ in each group. Values represent the mean $\pm$ S.D. ($n = 10$ for Con; $n = 6$ for treatment groups). Significant differences by one-way ANOVA and Tukey's test are indicated with # $P < 0.05$.

Figure 7. Pathological findings in each group of *Ppara*-null (KO) mice with PON

Low-magnification histological photographs of the tubular area stained with periodic acid Schiff (PAS). Scale bar=50 μm (KO + Veh, vehicle-treated *Ppara*-null mice; KO + Veh + PON, vehicle-treated *Ppara*-null mice with PON; KO + Los + PON, Los-treated *Ppara*-null mice with PON; KO + Irbe + PON, Irbe-treated *Ppara*-null mice with PON).