European Journal of Pharmacology 682 (2012) 131–136

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

Reduction of renal lipid content and proteinuria by a PPAR-γ agonist in a rat model of angiotensin II-induced hypertension

Aiko Sakamoto, Makiko Hongo, Kan Saito, Ryozo Nagai, Nobukazu Ishizaka *

Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form 9 February 2012
Accepted 15 February 2012
Available online 24 February 2012

Keywords:
Kidney
Lipid accumulation
Proteinuria
Angiotensin II
Peroxisome proliferator activated receptor

ABSTRACT

An excess of lipids may accumulate in the kidney in conditions such as diabetes and hypertension, and can potentially cause renal injury. We previously reported that an infusion of angiotensin II into a rat induced deposition of lipids in the renal tubular epithelial cells. Here we have examined the effect of pioglitazone, an agonist of the peroxisome proliferator-activated receptor-γ (PPAR-γ), on renal lipid accumulation and renal injury induced by angiotensin II infusion. Pioglitazone treatment (2.5 mg/kg/day) reduced the amount of triglycerides in the kidney of the angiotensin II-induced hypertensive rat without significantly altering either blood pressure levels or mRNA expression of lipogenic genes in the kidney. In addition, pioglitazone, either alone or in conjunction with angiotensin II, increased the expression of phosphorylated, but not total, AMP-activated protein kinase (AMPK). Proteinuria and kidney weight in the angiotensin II-infused rat were significantly decreased by pioglitazone treatment. In addition, pioglitazone suppressed iron deposition and ferritin protein induction, but did not alter upregulated expression of the antioxidative molecule, heme oxygenase-1, in the kidney of the angiotensin II-infused rat. These findings suggested that pioglitazone suppressed the angiotensin II-induced increase in renal lipid content by inhibiting its proteinuric action, but not by direct alteration of the expression or activity of lipid metabolism-related genes. Reduction of lipotoxic renal damage may represent one of the renoprotective effects provided by pioglitazone in hypertension with activation of the renin–angiotensin system.

1. Introduction

In an insulin resistant state and diabetes, excess lipid may accumulate in non-adipose tissue, such as the heart (Marfella et al., 2009), liver (Cusi, 2009), and pancreas (van Raalte et al., 2010), and may cause histological and functional injury to these tissues, termed lipotoxicity. Accumulation of lipids in the kidney is also known to occur in humans (Katsoulieris et al., 2010; Moorhead et al., 1982) and in animal models of diabetes (Lemieux et al., 1984), obesity (Dominguez et al., 2007), and aging (Jiang et al., 2005). Although the precise mechanism of renal lipid accumulation has not been completely elucidated, it may be a consequence of the increased reabsorption of filtered fatty acids bound to albumin (extracellular source) and/or increased synthesis or decreased oxidation of fatty acids in renal cells (intracellular source) (Sun et al., 2002; Thomas and Schreiner, 1993; Weinberg, 2006). The physiological importance of renal lipid accumulation, which is supported by the finding that transfer of a lipogenic gene into kidney results in an increase in both proteinuria and expression of transforming growth factor β1 (Sun et al., 2002), may be a consequence of the enhanced generation of reactive oxygen species (Kamijo et al., 2007), the disruption of intracellular signaling pathways, or the upregulation of proinflammatory and profibrotic factors (Bobulescu, 2010).

Administration of angiotensin II is reported to enhance insulin resistance (Ogihara et al., 2002) and to increase urinary protein excretion in animal models (Aizawa et al., 2000). We have previously reported that 7-day administration of angiotensin II to rats caused marked lipid accumulation in the tubular epithelial cells, where both enhanced superoxide generation and upregulation of fibroproliferative gene expression were observed (Ishizaka et al., 2006; Saito et al., 2005). Recent studies showed that peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists, which may promote adipogenesis and reduce ectopic lipid deposition, may be effective in reducing proteinuria by a mechanism independent of their hypoglycemic property (Benigni et al., 2006; Panchapakesan et al., 2005; Yotsumoto et al., 2003). To this end, here we have investigated the effect of the PPAR-γ agonist pioglitazone on renal lipid deposition and proteinuria in a rat model of angiotensin II-induced hypertension.

* Corresponding author at: Department of Cardiology, Osaka Medical College, Takatsuki-shi Daigaku-machi 2-7, Osaka 569-8686, Japan. Tel.: +81 72 683 1221; fax: +81 72 684 6533.
E-mail addresses: nobuishizka-tky@umin.ac.jp, ishizaka@poh.osaka-med.ac.jp (N. Ishizaka).

0014-2999/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2012.02.027

2. Materials and methods

2.1. Animal models

The experiments were performed in accordance with the guidelines for animal experimentation approved by the Animal Center for Biomedical Research, Faculty of Medicine, University of Tokyo. Angiotensin II-induced hypertension was induced in male Sprague–Dawley rats (250 to 300 g) by subcutaneous implantation of an osmotic minipump (Alzet model 2001; Alza Pharmaceutical, Vacaville, CA) as described previously (Aizawa et al., 2000). Briefly, Val5-Angiotensin II (Sigma, St. Louis, MO) was infused at doses of 0.7 mg/kg/day via subcutaneously implanted osmotic minipump for 7 consecutive days. In some angiotensin II-infused rats, pioglitazone (Takeda Pharmaceutical Co., Tokyo, Japan) was given orally at a dose of 2.5 mg/kg/day. Blood pressure, serum levels of lipids and insulin, and plasma levels of glucose, which were obtained from at least 7 samples in each group, have been described elsewhere (Sakamoto et al., in press).

2.2. Measurement of lipid contents in the kidney

For the measurement of tissue lipid content, 0.2 g of a tissue sample of renal cortex was homogenized with 4 mL of chloroform-methanol (1:2 by volume) using a homogenizer (Polytron; Kinematica, Lucerne, Switzerland), vigorously vortexed for 5 min, centrifuged at 3000 rpm for 5 min to separate the phases. Next, the lower phase obtained by centrifugation was evaporated under the stream of nitrogen gas, and then the lipid extract was dissolved in isopropanol. Total cholesterol and triglycerides were assayed using Total Cholesterol E Test Wako (Wako Pure Chemicals, Osaka, Japan) and Triglycerides E Test Wako (Wako Pure Chemicals), respectively.

2.3. Histological analysis

Oil red O staining was performed on sections of unfixed, freshly frozen kidney samples (3 μm in thickness). The areas of lipid deposition were calculated by using the image analysis software, Photoshop (Adobe Systems, San Jose, CA), and semiquantification of the lipid deposition was performed as described elsewhere (Ishizaka et al., 2006).

2.4. Western blot analysis

Western blot analysis was performed as described previously (Aizawa et al., 2000). Antibodies against total and phosphorylated forms of AMP-activated protein kinase (Cell Signaling Technology, Danvers, MA), and total and phosphorylated forms of acetyl-CoA carboxylase (ACC) (Cell Signaling Technology) were used at a dilution of 1/1000. Polyclonal antibodies against rat ferritin (Panapharm, Kumamoto, Japan) and heme oxygenase-1 (HO-1, StressGen, Victoria, BC, Canada), and monoclonal antibody against β-actin (Sigma) were used at dilutions of 1/2000, 1/2000, and 1/1000. The ECL Western blotting system (Amersham Life Sciences, Arlington Heights, IL) was used for detection. Bands were visualized by a lumino-analyzer (Fuji Photo Film, Tokyo, Japan). Band intensity was calculated and is expressed as a percentage of the control value.

2.5. Real time reverse transcription-polymerase chain reaction (RT-PCR)

Expression of lipid metabolism-related gene mRNA was analyzed by real time quantitative PCR performed by LightCycler together with hybriprobe technology (Roche Diagnostics, Basel, Switzerland). Expression of target genes was normalized to the mRNA expression of endogenous control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The target genes were as follows: sterol regulatory element-binding proteins (SREBP)-1c, fatty acid synthase (FAS), PPAR-γ, and PPAR-α. The forward and backward primers used are described elsewhere (Hongo et al., 2009).

2.6. Statistical analysis

Data are expressed as the mean ± S.E.M. We used ANOVA followed by a multiple comparison test to compare raw data, before expressing the results as a percentage of the control value using the statistical analysis software SPSS Dr II. A value of $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Characteristics of the experimental animals

Systolic blood pressure was not significantly different from controls in rats treated with pioglitazone alone, and systolic blood pressure in angiotensin II-treated rats, which was significantly higher than that in control rats, was lowered the concomitant treatment with pioglitazone slightly, albeit not significantly (Sakamoto et al., in press). Pioglitazone suppressed angiotensin II-induced increases in serum triglycerides ($P<0.05$) and in plasma glucose level ($P<0.05$) (data not shown). Pioglitazone also suppressed angiotensin II-induced increase in urinary protein excretion; however, pioglitazone did not improve the decreased creatinine clearance induced by angiotensin II (Fig. 1).

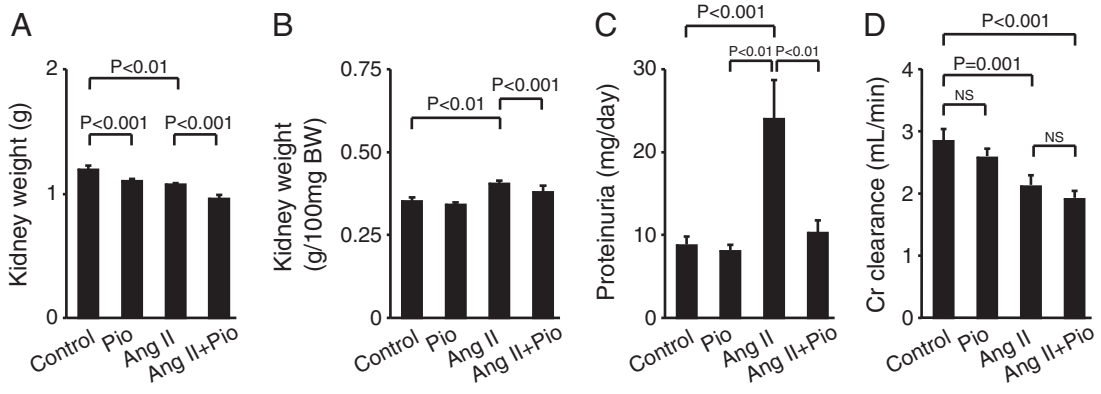

Fig. 1. Weight of kidney, extent of proteinuria, and creatinine (Cr) clearance. Data represent the mean ± S.E.M. of the results from 5 to 7 rats in each group. Ang II, angiotensin II; and Pio, pioglitazone.

3.2. Accumulation of lipids in the kidney

In the kidney sections, oil red O staining showed no apparent lipid deposition in the kidney of untreated rats or rats treated with pioglitazone alone (Fig. 2A, B). An increase in oil red O-positive lipid droplets was observed in the tubular epithelial cells in angiotensin II-infused rats (Fig. 2C), and this increase was suppressed by pioglitazone (Fig. 2D, E). Tissue content of triglycerides was found to be increased in the kidney of angiotensin II-infused rat, and this increase was again suppressed by pioglitazone (Fig. 3).

3.3. Regulation of genes related to lipid metabolism

As compared with untreated control rats ($n=6$), angiotensin II ($n=6$) significantly increased the expression of SREBP-1c mRNA ($187\pm25\%$, $P<0.01$ versus control) and FAS mRNA ($172\pm28\%$, $P<0.05$ versus control), and concomitant pioglitazone treatment ($n=5$) did not significantly suppress the angiotensin II-induced upregulation of these lipogenic genes: SREBP-1c ($210\pm36\%$, $P<0.01$ versus control) and FAS ($175\pm17\%$, $P<0.01$ versus control). Expression of PPAR-γ mRNA was not altered by angiotensin II ($85\pm14\%$, NS versus control) or angiotensin II plus pioglitazone ($105\pm17\%$, NS versus control). Angiotensin II downregulated the expression of PPAR- α mRNA ($59.5\pm8.8\%$, $P<0.05$ versus control), and this downregulation was inhibited by pioglitazone ($83.7\pm9.4\%$, NS versus control).

Angiotensin II increased the amount of phosphorylated AMPKα and ACC, although it did not significantly alter the total amount of AMPKα and ACC proteins. Pioglitazone treatment also increased phosphorylated AMPKα levels (Fig. 4).

3.4. Tissue iron deposition and protein expression of ferritin and HO-1

Angiotensin II caused iron deposition in the proximal tubular epithelial cells and upregulated the expression of HO-1 and ferritin protein (Fig. 5), as has been described previously (Ishizaka et al., 2002). The angiotensin II-induced iron deposition and ferritin induction was suppressed by pioglitazone. On the other hand, pioglitazone did not significantly reduce the upregulated HO-1 expression in the kidney of rats given angiotensin II.

4. Discussion

In the present study, we demonstrated that pioglitazone reduced the renal lipid content in the angiotensin II-infused rat, but did not significantly affect blood pressure levels. Pioglitazone reduced the extent of proteinuria, but did not significantly affect the angiotensin II-induced upregulation in lipogenic gene expression, suggesting that the reduction of lipid content in the tubular epithelial cells may be a consequence of a reduction of circulation lipids and/or a reduction of proteinuria. Pioglitazone also suppressed the deposition of iron in the tubular epithelial cells, which may, in conjunction with the reduction of lipid accumulation, act favorably to maintain normal tubular function.

Until now, several mechanisms have been postulated to play a role in the accumulation of lipids in tubular epithelial cells. The vast majority of plasma free fatty acids is carried on albumin (Richieri and Kleinfeld, 1995). Fatty acids bound to albumin are filtered through glomeruli and then reabsorbed from the filtrate via endocytosis by interacting with several membrane proteins and scaffolding and

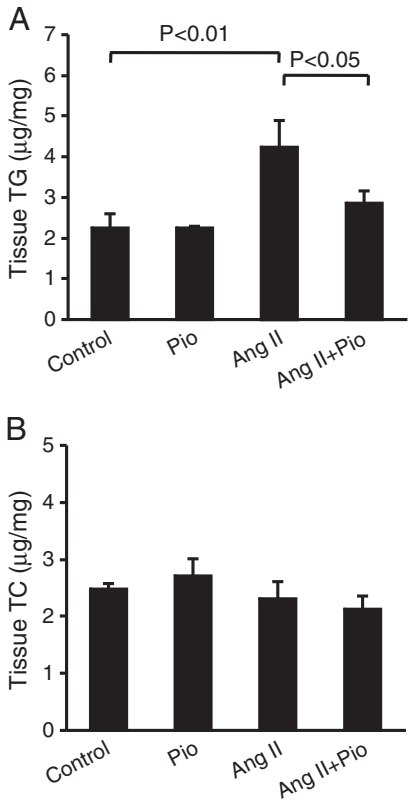

Fig. 3. Tissue content of lipids. Shown is the content of triglycerides (TG) (A) and total cholesterol (TC) (B) in the kidney. Data represent the mean ± S.E.M. of the results from 5 to 7 rats in each group. Abbreviations are the same as in Fig. 2.

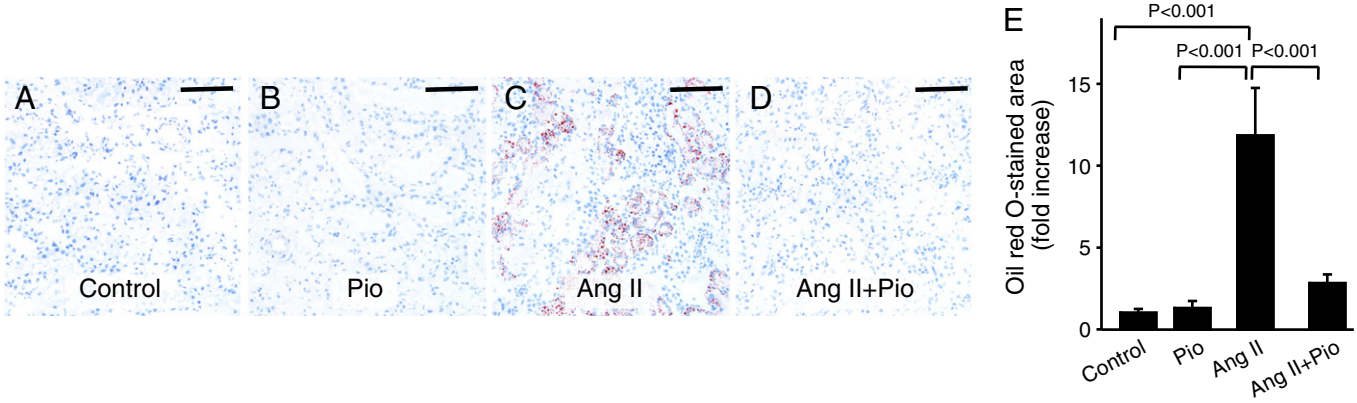

Fig. 2. Oil red O staining of the kidney of rats given angiotensin II with or without pioglitazone. Shown are kidney sections from a control rat (A), a rat given pioglitazone (B), a rat given angiotensin II (C), and a rat given angiotensin II plus pioglitazone (D). Original magnification, x 200. Scale bar indicates 100 μm. E. Semiquantification of the oil red O-stained area. Data represent the mean ± S.E.M. of the results from 4 to 5 rats in each group. Scale bar indicates 100 μm. Abbreviations are the same as in Fig. 1.

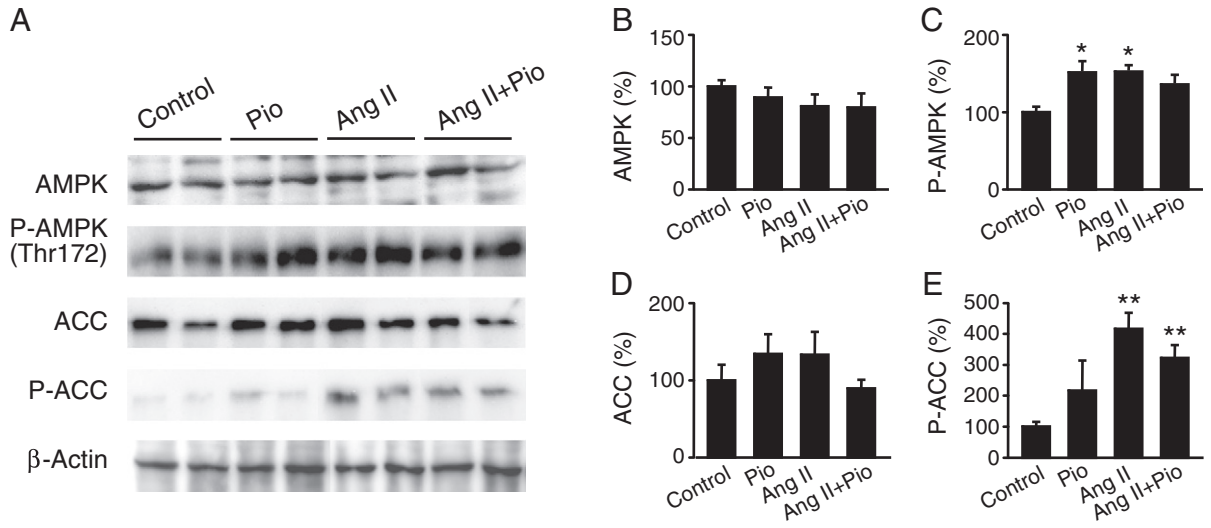

Fig. 4. Western blot analysis of AMP-activated protein kinase α (AMPK), acetyl-CoA carboxylase (ACC), phosphorylated (activated) AMPKα (P-AMPK), and phosphorylated (inactivated) ACC (P-ACCand). A. Representative blots. B, C, D, E. Protein expression in treated rats relative to control rats. Data represent the mean ± S.E.M. of the results from 4 to 6 rats in each group. $^{*}P<0.05$ $^{**}P<0.01$ versus control group. Abbreviations are the same as in Fig. 1.

regulatory molecules (Pollock and Poronnik, 2007), before being transported into tubular epithelial cells. Free fatty acid delivered to the tubular cells in excess can be deposited as triglycerides in intracellular lipid droplets. It has been demonstrated that renal lipid accumulation may be induced by the regulation of lipogenic gene expression (Sun et al., 2002); however, it may also be induced by an excess supply of lipids (Bobulescu, 2010). Of note, proteinuric toxicity leading to tubulointerstitial injury is generally thought to be caused by macromolecules that are filtered through glomeruli including fatty acids (Chen et al., 1998; Kamijo et al., 2002; Ong and Moorhead, 1994).

Several previous studies have demonstrated the antisteatotic action of PPAR-γ agonists in the heart, liver, and skeletal muscle (Hockings et al., 2003; Rasouli et al., 2005; Zhou et al., 2000); however, only a few studies have reported the effect of PPAR-γ agonists on the renal lipid content. Bobulescu et al. reported that the PPAR-γ agonist reduces renal cortical lipid content in a rat model of metabolic syndrome (Bobulescu et al., 2009). They also found that PPAR-γ agonist did not affect intracellular lipid content in cultured renal cells possessing characteristics of the renal proximal tubules (OKP cells), suggesting that the direct effect of PPAR-γ activation in the tubular epithelium does not account for the antisteatotic effect of this agent in the kidney of the metabolic syndrome rat. Sui et al. found that elevation of serum triglycerides levels and fat deposits in the renal inner cortex could be seen in the uninephrectomized rat, and both of these phenomena were suppressed by treatment of the uninephrectomized rat with angiotensin-converting enzyme inhibitor (ACEI) (Sui et al., 2010). They also found an increase in PPAR-γ immunoreactivity in the kidney

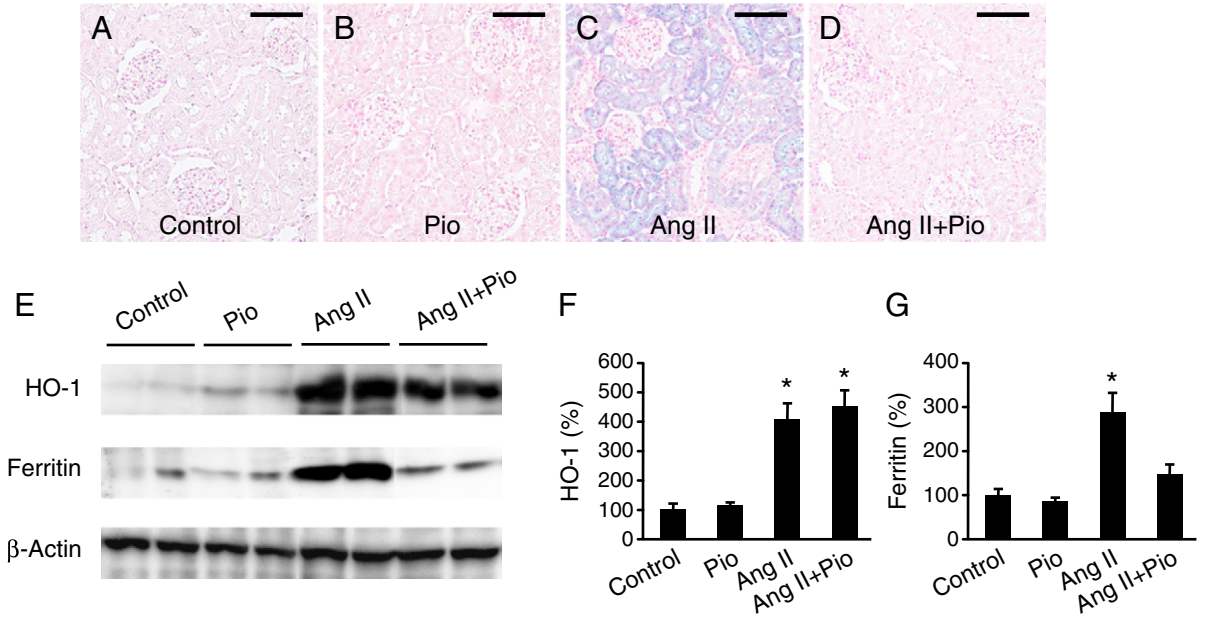

Fig. 5. Iron deposition and protein expression of heme oxygenase-1 (HO-1) and ferritin. A-D. Prussian blue staining. Shown are kidney sections from a control rat (A), a rat given pioglitazone (B), a rat given angiotensin II (C), and a rat given angiotensin II plus pioglitazone (D). Original magnification, x 200. Scale bar indicates 100 μm. E. Representative Western blots of HO-1 and ferritin. F, G. HO-1 and ferritin expression in treated rats relative to control rats. Data represent the mean ± S.E.M. of the results from 4 to 6 rats in each group. Scale bar indicates 100 μm. $^{*}P<0.001$ versus control group. Abbreviations are the same as in Fig. 1.

of uninephrectomized rat, which was again suppressed by ACEI treatment. In Sui et al.'s study, however, it was not clear whether increased PPAR-γ expression is a cause or a result of renal lipid accumulation.

In the current study, we did not find evidence suggesting that pioglitazone modulated the angiotensin II-induced upregulation of lipogenic gene expression (Saito et al., 2005). In addition, the amount of phosphorylated AMPK was not changed by pioglitazone treatment. Taking these results together, the tubular accumulation of lipids and iron may be the consequence of angiotensin II-induced glomerular damage and subsequent reabsorption of the glomerular filtrate into tubular cells. On the other hand, an excess amount of lipids (Bobulescu et al., 2009) or iron (Ishizaka et al., 2002) in the tubular cells may further enhance tubulointerstitial damage. Considering that pioglitazone may exert anti-inflammatory effects on tubular epithelial cells (Li et al., 2005), pioglitazone treatment may act favorably on the renal tubules in a rat model of angiotensin II-induced hypertension.

It has been shown in several previous studies that HO-1 may act favorably on the kidney through its anti-oxidative and anti-inflammatory properties. We found that pioglitazone treatment did not reduce the angiotensin II-induced upregulation of HO-1 (Fig. 5). We previously reported that expression of HO-1, but not ferritin, was induced by angiotensin II in the proximal tubular epithelial cells in vitro (Aizawa et al., 2000; Ishizaka et al., 2002). It has recently been shown that thiazolidinediones upregulate, albeit in non-renal cells, HO-1 expression via transcriptional activation (Kronke et al., 2007). On the other hand, however, considering that pioglitazone alone did not upregulate renal HO-1 expression, preservation of the upregulation of HO-1 expression might be explained by the angiotensin II-induced HO-1 upregulation in proximal tubular epithelial cells in the current study.

An anti-proteinuric effect of pioglitazone has been shown in several different animal models, including models of obese diabetes (Yoshimoto et al., 1997), metabolic syndrome (Ohtomo et al., 2007), immune-mediated glomerulonephritis (Benigni et al., 2006), and hypercholesterolemia (Omasu et al., 2007). Several mechanisms have been postulated to underlie pioglitazone-induced renoprotection, such as antihypertensive effect (Yoshimoto et al., 1997) and upregulation of nephrin, the main structural protein of the slit diaphragm (Benigni et al., 2006). Considering that inhibition of the renin–angiotensin system is also effective in reducing proteinuria in these animal models (Kong et al., 2011; Namikoshi et al., 2008), it is suggested that amelioration of tubular lipotoxicity might represent pioglitazone-induced amelioration of glomerular and tubulointerstitial injury mediated, in part, by activation of the renin–angiotensin system.

Although angiotensin II infusion increases PPAR-α expression in the heart (Hongo et al., 2009), here PPAR-α expression was found to be decreased in the angiotensin II-infused rat kidney. PPAR-α is suggested to act renoprotectively cells against fatty acid toxicity associated with proteinuria (Kamijo et al., 2007) or induced by diet (Shin et al., 2009). In addition, PPAR-α gene deficiency worsened albuminuria in diabetic animals (Park et al., 2006a) and PPAR-α agonists have been shown to reduce proteinuria in various animal models with renal injury (Hou et al., 2010; Park et al., 2006b; Zhou et al., 2011). Although the mechanism underlying angiotensin II-induced PPAR-α downregulation in the kidney remains to be elucidated, it is possible that pioglitazone-induced recovery of PPAR-α expression in the angiotensin II-infused rat kidney might have also acted favorably in reducing proteinuria.

In summary, in the rat model of angiotensin II-induced hypertension, pioglitazone reduced renal deposition of lipids as well as iron, which was not accompanied by an alteration in the angiotensin II-induced upregulation of lipogenic gene expression. These effects of pioglitazone may act favorably against renal tubular injury induced by lipotoxicity and oxidative stress occurring in the angiotensin II-infused rat.

Acknowledgements

This work was supported by Grants in Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (Grant 19590937) and Grant from the Takeda Science Foundation, the Sankyo Foundation of Life Science, Okinaka Memorial Institute for Medical Research, and Daiwa Securities Health Foundation.

References

Aizawa, T., Ishizaka, N., Taguchi, J., Nagai, R., Mori, I., Tang, S.S., Ingelfinger, J.R., Ohno, M., 2000. Heme oxygenase-1 is upregulated in the kidney of angiotensin II-induced hypertensive rats: possible role in renoprotection. Hypertension 35, 800–806.

Benigni, A., Zoja, C., Tomasoni, S., Campana, M., Corna, D., Zanchi, C., Gagliardini, E., Garofano, E., Rottoli, D., Ito, T., Remuzzi, G., 2006. Transcriptional regulation of nephrin gene by peroxisome proliferator-activated receptor-gamma agonist: molecular mechanism of the antiproteinuric effect of pioglitazone. J. Am. Soc. Nephrol. 17, 1624–1632.

Bobulescu, I.A., 2010. Renal lipid metabolism and lipotoxicity. Curr. Opin. Nephrol. Hypertens. 19, 393–402.

Bobulescu, I.A., Dubree, M., Zhang, J., McLeroy, P., Moe, O.W., 2009. Reduction of renal triglyceride accumulation: effects on proximal tubule Na+/H+ exchange and urinary acidification. Am. J. Physiol. Ren. Physiol. 297, F1419–F1426.

Chen, L., Boadle, R.A., Harris, D.C., 1998. Toxicity of holotransferrin but not albumin in proximal tubule cells in primary culture. J. Am. Soc. Nephrol. 9, 77–84.

Cusi, K., 2009. Nonalcoholic fatty liver disease in type 2 diabetes mellitus. Curr. Opin. Endocrinol. Diab. Obes. 16, 141–149.

Dominguez, J., Wu, P., Packer, C.S., Temm, C., Kelly, K.J., 2007. Lipotoxic and inflammatory phenotypes in rats with uncontrolled metabolic syndrome and nephropathy. Am. J. Physiol. Ren. Physiol. 293, F670–F679.

Hockings, P.D., Changani, K.K., Saeed, N., Reid, D.G., Birmingham, J., O'Brien, P., Osborne, J., Toseland, C.N., Buckingham, R.E., 2003. Rapid reversal of hepatic steatosis, and reduction of muscle triglyceride, by rosiglitazone: MRI/S studies in Zucker fatty rats. Diab. Obes. Metab. 5, 234–243.

Hongo, M., Ishizaka, N., Furuta, K., Yahagi, N., Saito, K., Sakurai, R., Matsuzaki, G., Koike, K., Nagai, R., 2009. Administration of angiotensin II, but not catecholamines, induces accumulation of lipids in the rat heart. Eur. J. Pharmacol. 604, 87–92.

Hou, X., Shen, Y.H., Li, C., Wang, F., Zhang, C., Bu, P., Zhang, Y., 2010. PPARalpha agonist fenofibrate protects the kidney from hypertensive injury in spontaneously hypertensive rats via inhibition of oxidative stress and MAPK activity. Biochem. Biophys. Res. Commun. 394, 653–659.

Ishizaka, N., Aizawa, T., Yamazaki, I., Usui, S., Mori, I., Kurokawa, K., Tang, S.S., Ingelfinger, J.R., Ohno, M., Nagai, R., 2002. Abnormal iron deposition in renal cells in the rat with chronic angiotensin II administration. Lab. Investig. 82, 87–96.

Ishizaka, N., Matsuzaki, G., Saito, K., Noiri, E., Mori, I., Nagai, R., 2006. Expression and localization of PDGF-B, PDGF-D, and PDGF receptor in the kidney of angiotensin II-infused rat. Lab. Investig. 86, 1285–1292.

Jiang, T., Wang, Z., Proctor, G., Moskowitz, S., Liebman, S.E., Rogers, T., Lucia, M.S., Li, J., Levi, M., 2005. Diet-induced obesity in C57BL/6J mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. J. Biol. Chem. 280, 32317–32325.

Kamijo, A., Kimura, K., Sugaya, T., Yamanouchi, M., Hase, H., Kaneko, T., Hirata, Y., Goto, A., Fujita, T., Omata, M., 2002. Urinary free fatty acids bound to albumin aggravate tubulointerstitial damage. Kidney Int. 62, 1628–1637.

Kamijo, Y., Hora, K., Kono, K., Takahashi, K., Higuchi, M., Ehara, T., Kiyosawa, K., Shigematsu, H., Gonzalez, F.J., Aoyama, T., 2007. PPARalpha protects proximal tubular cells from acute fatty acid toxicity. J. Am. Soc. Nephrol. 18, 3089–3100.

Katsoulieris, E., Mabley, J.G., Samai, M., Sharpe, M.A., Green, I.C., Chatterjee, P.K., 2010. Lipotoxicity in renal proximal tubular cells: relationship between endoplasmic reticulum stress and oxidative stress pathways. Free Radic. Biol. Med. 48, 1654–1662.

Kong, X., Zhang, D.Y., Wu, H.B., Li, F.X., 2011. Losartan and pioglitazone ameliorate nephropathy in experimental metabolic syndrome rats. Biol. Pharm. Bull. 34, 693–699.

Kronke, G., Kadl, A., Ikonomu, E., Bluml, S., Furnkranz, A., Sarembock, I.J., Bochkov, V.N., Exner, M., Binder, B.R., Leitinger, N., 2007. Expression of heme oxygenase-1 in human vascular cells is regulated by peroxisome proliferator-activated receptors. Arterioscler. Thromb. Vasc. Biol. 27, 1276–1282.

Lemieux, G., Moulin, B., Davignon, J., Huang, Y.S., 1984. The lipid content of the diabetic kidney of the rat. Can. J. Physiol. Pharmacol. 62, 1274–1278.

Li, H., Ruan, X.Z., Powis, S.H., Fernando, R., Mon, W.Y., Wheeler, D.C., Moorhead, J.F., Varghese, Z., 2005. EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. Kidney Int. 67, 867–874.

Marfella, R., Di Filippo, C., Portoghese, M., Barbieri, M., Ferraraccio, F., Siniscalchi, M., Cacciapuoti, F., Rossi, F., D'Amico, M., Paolisso, G., 2009. Myocardial lipid accumulation in patients with pressure-overloaded heart and metabolic syndrome. J. Lipid Res. 50, 2314–2323.

Moorhead, J.F., Chan, M.K., El-Nahas, M., Varghese, Z., 1982. Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. Lancet 2, 1309–1311.

Namikoshi, T., Tomita, N., Satoh, M., Haruna, Y., Kobayashi, S., Komai, N., Sasaki, T., Kashihara, N., 2008. Pioglitazone enhances the antihypertensive and renoprotective effects of candesartan in Zucker obese rats fed a high-protein diet. Hypertens. Res. 31, 745–755.

Ogihara, T., Asano, T., Ando, K., Chiba, Y., Sakoda, H., Anai, M., Shojima, N., Ono, H., Onishi, Y., Fujishiro, M., Katagiri, H., Fukushima, Y., Kikuchi, M., Noguchi, N., Aburatani, H., Komuro, I., Fujita, T., 2002. Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. Hypertension 40, 872–879.

Ohtomo, S., Izuhara, Y., Takizawa, S., Yamada, N., Kakuta, T., van Ypersele de Strihou, C., Miyata, T., 2007. Thiazolidinediones provide better renoprotection than insulin in an obese, hypertensive type II diabetic rat model. Kidney Int. 72, 1512–1519.

Omasu, F., Oda, T., Yamada, M., Yoshizawa, N., Yamakami, K., Sakurai, Y., Miura, S., 2007. Effects of pioglitazone and candesartan on renal fibrosis and the intrarenal plasmin cascade in spontaneously hypercholesterolemic rats. Am. J. Physiol. Ren. Physiol. 293, F1292–F1298.

Ong, A.C., Moorhead, J.F., 1994. Tubular lipidosis: epiphenomenon or pathogenetic lesion in human renal disease? Kidney Int. 45, 753–762.

Panchapakesan, U., Chen, X.M., Pollock, C.A., 2005. Drug insight: thiazolidinediones and diabetic nephropathy—relevance to renoprotection. Nat. Clin. Pract. Nephrol. 1, 33–43.

Park, C.W., Kim, H.W., Ko, S.H., Chung, H.W., Lim, S.W., Yang, C.W., Chang, Y.S., Sugawara, A., Guan, Y., Breyer, M.D., 2006a. Accelerated diabetic nephropathy in mice lacking the peroxisome proliferator-activated receptor alpha. Diabetes 55, 885–893.

Park, C.W., Zhang, Y., Zhang, X., Wu, J., Chen, L., Cha, D.R., Su, D., Hwang, M.T., Fan, X., Davis, L., Striker, G., Zheng, F., Breyer, M., Guan, Y., 2006b. PPARalpha agonist fenofibrate improves diabetic nephropathy in db/db mice. Kidney Int. 69, 1511–1517.

Pollock, C.A., Poronnik, P., 2007. Albumin transport and processing by the proximal tubule: physiology and pathophysiology. Curr. Opin. Nephrol. Hypertens. 16, 359–364.

Rasouli, N., Raue, U., Miles, L.M., Lu, T., Di Gregorio, G.B., Elbein, S.C., Kern, P.A., 2005. Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue. Am. J. Physiol. Endocrinol. Metab. 288, E930–E934.

Richieri, G.V., Kleinfeld, A.M., 1995. Unbound free fatty acid levels in human serum. J. Lipid Res. 36, 229–240.

Saito, K., Ishizaka, N., Hara, M., Matsuzaki, G., Sata, M., Mori, I., Ohno, M., Nagai, R., 2005. Lipid accumulation and transforming growth factor-beta upregulation in the kidneys of rats administered angiotensin II. Hypertension 46, 1180–1185.

Sakamoto, A., Hongo, M., Furuta, K., Saito, K., Nagai, R., Ishizaka, N., in press. Pioglitazone ameliorates systolic and diastolic cardiac dysfunction in rat model of angiotensin II-induced hypertension. Int. J. Cardiol.

Shin, S.J., Lim, J.H., Chung, S., Youn, D.Y., Chung, H.W., Kim, H.W., Lee, J.H., Chang, Y.S., Park, C.W., 2009. Peroxisome proliferator-activated receptor-alpha activator fenofibrate prevents high-fat diet-induced renal lipotoxicity in spontaneously hypertensive rats. Hypertens. Res. 32, 835–845.

Sui, Y., Zhao, H.L., Fan, R.R., Guan, J., He, L., Lee, H.M., Chan, J.C., Tong, P.C., 2010. Renin–angiotensin system activation in renal adipogenesis. Am. J. Physiol. Ren. Physiol. 298, F391–F400.

Sun, L., Halaihel, N., Zhang, W., Rogers, T., Levi, M., 2002. Role of sterol regulatory element-binding protein 1 in regulation of renal lipid metabolism and glomerulosclerosis in diabetes mellitus. J. Biol. Chem. 277, 18919–18927.

Thomas, M.E., Schreiner, G.F., 1993. Contribution of proteinuria to progressive renal injury: consequences of tubular uptake of fatty acid bearing albumin. Am. J. Nephrol. 13, 385–398.

van Raalte, D.H., van der Zijl, N.J., Diamant, M., 2010. Pancreatic steatosis in humans: cause or marker of lipotoxicity? Curr. Opin. Clin. Nutr. Metab. Care 13, 478–485.

Weinberg, J.M., 2006. Lipotoxicity. Kidney Int. 70, 1560–1566.

Yoshimoto, T., Naruse, M., Nishikawa, M., Naruse, K., Tanabe, A., Seki, T., Imaki, T., Demura, R., Aikawa, E., Demura, H., 1997. Antihypertensive and vasculo- and renoprotective effects of pioglitazone in genetically obese diabetic rats. Am. J. Physiol. 272, E989–E996.

Yotsumoto, T., Naitoh, T., Kanaki, T., Matsuda, M., Tsuruzoe, N., 2003. A novel peroxisome proliferator-activated receptor (PPAR)gamma agonist, NIP-222, reduces urinary albumin excretion in streptozotocin-diabetic mice independent of PPARgamma activation. Metabolism 52, 1633–1637.

Zhou, Y.T., Grayburn, P., Karim, A., Shimabukuro, M., Higa, M., Baetens, D., Orci, L., Unger, R.H., 2000. Lipotoxic heart disease in obese rats: implications for human obesity. Proc. Natl. Acad. Sci. U. S. A. 97, 1784–1789.

Zhou, Y., Kong, X., Zhao, P., Yang, H., Chen, L., Miao, J., Zhang, X., Yang, J., Ding, J., Guan, Y., 2011. Peroxisome proliferator-activated receptor-alpha is renoprotective in doxorubicin-induced glomerular injury. Kidney Int. 79, 1302–1311.