Yonsei Medical Journal
Vol. 48, No. 2, pp. 308 - 316, 2007

Induction of PPAR Gamma mRNA and Protein Expression by Rosiglitazone in Chronic Cyclosporine Nephropathy in the Rat

Kyung Ohk Ahn,[1] Sun Woo Lim,[1] Hyun Joo Yang,[1] Can Li,[1,2] Akira Sugawara,[3] Sadayoshi Ito,[3] Bum Soon Choi,[1] Yong Soo Kim,[1] Jin Kim,[4] and Chul Woo Yang[1]

[1]*Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Seoul, Korea;*
[2]*Department of Internal Medicine, The Affiliated Hospital, YanBian University Medical College, YanJi 133000, JiLin, PR China;*
[3]*Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan;*
[4]*Cell Death Research Center, Department of Anatomy, The Catholic University of Korea, Seoul, Korea.*

Purpose: We recently reported that rosiglitazone (RGTZ), a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, has a protective effect against cyclosporine (CsA)-induced renal injury. Here we report the effect of RGTZ on peroxisome proliferator-activated receptor gamma (PPARγ) expression in an experimental model of chronic cyclosporine (CsA) nephropathy. **Materials and Methods:** Chronic CsA nephropathy was induced in Sprague-Dawley rats by administering CsA (15 mg/kg per day) for 28 days, and control rats were treated with vehicle (VH group, olive oil 1 mL/kg per day) for 28 days. RGTZ (3 mg/kg) was concurrently administered via gavage to the CsA and VH groups. Expression of PPARγ mRNA and protein was evaluated with RT-PCR, immunohistochemistry, and immunoblotting. **Results:** PPARγ mRNA expression was similar to the level of PPARγ protein constitutively expressed in the kidneys of the VH treated rats, with expression in the glomerular epithelial, distal tubular cells, and collecting tubular cells. PPARγ protein expression in CsA-treated rat kidneys was significantly less than in the VH group. However, concomitant administration of RGTZ restored PPARγ protein expression in the kidneys of the CsA-reated rats. **Conclusion:** Exogenous administration of RGTZ treatment upregulates PPARγ expression and that this mechanism may play a role in protecting against CsA-induced renal injury.

Key Words: Cyclosporine, rosiglitazone, PPAR gamma, nephrotoxicity

Received June 8, 2006
Accepted August 24, 2006

This work was supported by the Korea Science & Engineering Foundation (KOSEF) (R13-2002-005-01001-0) through the Cell Death Disease Research Center at The Catholic University of Korea.

Reprint address: requests to Dr. Chul Woo Yang, Department of Internal Medicine, Kangnam St. Mary's Hospital, 505 Banpo-dong, Seocho-gu, Seoul 137-040, Korea. Tel: 82-2-590-2527, Fax: 82-2-536-0323, E-mail: yangch@catholic.ac.kr

INTRODUCTION

Chronic cyclosporine A (CsA) nephropathy is characterized by progressive renal failure and irreversible renal striped interstitial fibrosis, inflammatory cell infiltrations, and hyalinosis of the afferent glomerular arterioles.[1,2] Based on *in vitro* and *in vivo* studies, this pathology has been attributed to activation of the intrarenal renin- angiotensin system (RAS), increased release of endothelin-1, dysregulation of nitric oxide (NO) and NO synthases, an imbalance of prostaglandins and thromboxane, stimulation of the sympathetic nervous system, and upregulation of transforming growth beta-1 (TGF-β1) and osteopontin (OPN).[3,4]

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear hormone receptor superfamily of ligand-dependent transcription factors. PPAR plays an important role in the general transcriptional control of numerous cellular processes, including lipid metabolism, glucose homeostasis, cell cycle progression, cell differentiation, inflammation, and extracellular remodeling.[5] Three PPAR isoforms (PPARα, PPAR β, PPARγ) have been cloned and are differentially expressed in several tissues including the kidney. In the rat kidney, PPARγ protein is widely expressed along the nephron segments,[6] but its role in the kidney has not been determined.

Rosiglitazone (RGTZ), a PPARγ agonist, was originally developed as an anti-diabetic agent, but diverse actions of RGTZ have been reported. For example, RGTZ protects pancreatic function and prevents islet abnormality in Zucker fatty rats,[7] and has anti-inflammatory and anti-fibrotic effects in non-diabetic renal diseases.[5] In addition, RGTZ has protective effects against ischemic insults in the heart.[8,9]

We previously reported that RGTZ has a protective effect against CsA-induced renal injury,[10] but the mechanism behind this action is unclear. We evaluated the effects of RGTZ on PPARγ mRNA and protein expression in CsA-induced renal injury using the well-known experimental model of chronic CsA nephropathy. Our data demonstrate that RGTZ induces PPARγ mRNA and protein expression in the kidney. This response may explain the protective effects of RGTZ against CsA-induced renal injury.

MATERIALS AND METHODS

Animals and drugs

The Animal Care Committee of The Catholic University approved the experimental protocol. Male Sprague-Dawley rats (Charles River Technology, Seoul, Korea), initially weighing 230 to 250 g, were housed in cages (Nalge Co., Rochester, NY, USA) in a controlled temperature and light environment and allowed free access to a low salt diet (LSD 0.05% sodium, Teklad Premier, Madison, WI, USA) throughout the experimental period. CsA (Novartis Pharma Ltd., Basle, Switzerland) was diluted in olive oil (Sigma Co., Louise, MO, USA) to a final concentration of 15 mg/mL. RGTZ was dissolved in DMSO and given to the rats by oral gavage.

Antibody

To determine the expression of PPARγ in CsA treated-rat kidney, we used a specific rabbit polyclonal antibody against synthetic peptides based on mouse PPARγ amino acid residues.[6] These peptides were synthesized, conjugated to keyhole limpet hemocyanin via m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester, and used to immunize rabbits as previously described.[11] The PPARγ antibody used in this study recognized both PPARγ1 and PPARγ2.

Experimental design

Rats were randomized to four groups and treated for four weeks:

1) Vehicle group (VH, n = 6) in which rats received olive oil (1 mL/kg per day s.c.).
2) VH + RGTZ group (n = 6) in which rats received VH and RGTZ (3 mg/kg per day).
3) CsA group (n = 7) in which rats received CsA (15 mg/kg per day s.c.).
4) CsA + RGTZ group (n = 7) in which rats received both CsA and RGTZ (3 mg/kg per day).

The CsA dose (15 mg/kg) was chosen based on our previous findings.[10]

Measurement of basic parameters

After starting the treatment, rats were pair-fed and daily body weight (BW) was monitored. Systolic blood pressure (SBP) was recorded at the end of the study in conscious rats by the tail-cuff method with a plethysmography using a tail manometer-tachometer system (BP-2000, Visitech system, Apex, NC, USA), and at least three readings for each rat were averaged. Prior to sacrifice, animals were individually housed in metabolic cages (Tecniplast, Gazzada, Italy) for 24 h urine collection. The following day, animals were anesthetized with ketamine, and blood sample and tissue specimens were obtained. Urine protein (UP) and serum creatinine (Scr) were measured by a Cobas autoanalyzer (Roche Diagnotics, Div. Hoffman-La Roche Inc., Nutely., Switzerland). The creatinine clearance (Ccr) was calculated using a standard formula. Whole blood CsA level was measured by a monoclonal radioimmunoassay (Incstar, Stillwater, MN, USA).

Measurement of CsA-induced interstitial fibrosis

A finding of tubulointerstitial fibrosis (TIF) was defined as a matrix-rich expansion of the interstitium with tubular dilatation, tubular atrophy, tubular cast formation, sloughing of tubular

epithelial cells, or thickening of the tubular basement membrane. A minimum of 20 fields per section was assessed using a color image analyzer (TDI Scope Eye Version 3.0 for Windows, Olympus, Japan). Briefly, the image was captured, and the extent of TIF was quantified using the Polygon program by counting the percentage of areas injured per field of cortex under 100 × magnification as previously described.[12] Histopathological analyses were performed in randomly selected cortical fields of sections by a pathologist blinded to the identity of the treatment groups.

Immunohistochemistry for PPARγ protein

Harvested rat kidney tissues were embedded in wax. After dewaxing, four-micron sections were incubated with 0.5% Triton × 100 in phosphate-buffered saline (PBS) solution for 30 min and washed with PBS three times for 15 min. Non-specific binding sites were blocked with normal horse serum diluted 1 : 10 in 0.3 % bovine serum albumin for 30-60 min and then incubated for 2 h at 4℃ in mouse antiserum against PPAR. We detected PPARγ by using an anti-PPARγ antibody diluted in 1 : 1000 in a humid environment. After rinsing in Tris-buffered saline (TBS), sections were incubated with peroxidase-conjugated rabbit anti-mouse IgG (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for 30 min. Sections were stained with a mixture of 0.05% 3,3′-diaminobenzidine containing 0.01% H_2O_2 at room temperature until a brown color was visible, washed with TBS, and examined under light microscopy. Immunoreactivity of PPARγ protein expression was evaluated with 5 grades as described previously.[13]

Immunoblotting of PPARγ protein

For immunoblotting analysis, kidney-cortex tissue was homogenized in lysis buffer (20 mM/L Tris-Cl [pH 7.6], 150 mM/L NaCl, 1% [wt/vol] sodium deoxycholate, 1% [vol/vol] Triton X-100, 0.1% SDS, 2 mM/L NaVO3, and freshly added 1% [vol/vol] aprotinin, leupeptin [1 µg/mL], pepstatin [1 µg/mL], and 1 Mm/L phenylmethylsulflonyl-fluoride [PMSF]). Homogenates were centrifuged at 3000 rpm for 15 min at 4℃, and the protein concentration of the lysate was determined by a Bradford protein microassay (Bio-RAD, Laboratories, Hercules, CA, USA). Protein samples were resolved by 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then electroblotted onto Bio-Blot nitrocellulose membrane (Bio-RAD). The loading of an equal amount of protein (100 µg) was verified by Ponceau S staining. The membrane was blocked for 1 h in TBS-T (10 Mm Tri-CL, 150 Mm/L NaCl [pH 8.0], 0.05% tween-20) containing 5% nonfat powdered milk. We detected PPAR(by using an anti-PPAR(antibody diluted 1 : 1500 for 1 h. Primary antibody incubation was followed by six washes with TBS-T. The blot was then incubated with secondary antibody (Anti-rabbit Ig) conjugate at 1 : 1000 (Amersham Biosciences, Buckinghamshire, UK) for 1 h. Antibody-reactive protein was detected with enhanced chemiluminescence (ECL, Amersham Biosciences). We obtained optical densities using the VH group as a 100% reference and normalized with β-actin.

RNA extraction and RT-PCR

Total RNA was extracted using RNAzol reagent (TEL-TEST, Friendwood, TX, USA) according to the manufacturer's instructions. First-stand cDNA was reverse-transcribed from the RNA using random hexanucleotide primers. cDNA was synthesized using the First Stand Synthesis Kit for RT-PCR (Roche Diagnostics Scandinavia AB, Bromma, Sweden) using 1 µg of total RNA according to the manufacturer's instructions. The specific PCR primers used were: PPARγ, forward primer, 5′-TGATATCGACCAGCTGAACC-3′, reverse primer, 5′-GTCCTCCAGCTGTTCGCCA-3′; GAPDH, forward primer, 5′-AATGCATCCTGCA CCACCA A-3′, reverse primer, 5′-GATGCCATAT TCATTGT CATA-3′. The cDNA was then amplified using PCR with ten micromoles of each primer. The samples were first denatured at 94℃ for 3.5 min followed by 32 cycles of 94℃ for 1 min (melt), 58℃ for 1 min (anneal), and 72℃ for 1 min (extend). The last cycle was followed by additional extension of 8 min at 72℃.[14]

Statistical analysis

Data are expressed as mean ± SEM, and all

statistical analyses were conducted with SPSS version 9.0 (SPSS, Inc., Chicago, IL, USA). Multiple comparisons among groups were performed by one-way ANOVA with the post hoc Bonferroni test. Statistical significance was defined as $p < 0.05$.

RESULTS

Functional and histological studies

Table 1 shows basic parameters in each experimental group. BW was significantly less in the CsA group compared with the VH group. Concomitant treatment of RGTZ did not increase BW in the VH group but did increase BW in the CsA group. SBP and UPE was not different among study groups. The CsA group showed a significant an increase in Scr and decrease in Ccr compared with the VH group. Figure 1 shows the results of trichrome-staining in the four groups. There was no significant interstitial fibrosis in the VH (Fig. 1A) and VH + RGTZ (Fig. 1B) group. Rats treated with CsA had characteristic morphological features similar to the renal lesions associated with chronic CsA-induced nephrotoxicity in humans (Fig. 1C). Focal tubulointerstitial fibrosis, tubular atrophy, and mononuclear inflammatory cell infiltration were observed. However, the CsA + RGTZ group had less TIF the CsA group (Fig. 1D).

Table 1. Basic Parameters in Experimental Groups

Parameters	VH (n = 6)	VH + RGTZ (n = 6)	CsA (n = 7)	CsA + RGTZ (n = 7)
Body weight (g)	273 ± 1	272 ± 4	248 ± 3*	258 ± 3*
SBP (mmHg)	116 ± 8	118 ± 2	125 ± 3	121 ± 2
Scr (mg/dL)	0.69 ± 0.02	0.74 ± 0.02	1.21 ± 0.02*	0.89 ± 0.03†
Ccr (mL/min/100g)	0.51 ± 0.07	0.46 ± 0.07	0.18 ± 0.01*	0.31 ± 0.04†
UPE (mg/24hr)	8.56 ± 1.19	8.36 ± 1.20	8.04 ± 1.65	8.0 ± 0.72
CsA Conc (ng/mL)	0 ± 0	0 ± 0	1820 ± 50	1826 ± 34
TIF (%)	0 ± 0	0 ± 0	32.3.0 ± 1.7*	22.7 ± 0.8†

Values are means ± SE.
N, No. of rats; VH, vehicle-treated group; CsA, cyclosporine A; RGTZ, rosiglitazone; SBP, systolic blood pressure; Scr, serum creatinine; Ccr, creatinine clearance; UPE, proteinuria; TIF, tubulointerstitial fibrosis.
*$p < 0.05$ vs. VH or VH + RGTZ groups.
†$p < 0.05$ vs. CsA.

Table 2. Distribution Pattern of PPARγ in Each Group

	VH	VH + RGTZ	CsA	CsA + RGTZ
Podocyte	±	±	±	±
PT	−	+	−	−
DT	+	+++	±	++
CCD	+	++	±	+
IMCD	±	±	±	±

For each group, 3-4 animals were examined.
PPARγ, peroxisome proliferator activated receptor gamma; VH, vehicle-treated group; CsA, cyclosporine A; RGTZ, rosiglitazone. PT, proximal tubule; DT, distal tubule; CCD, cortical collecting duct; IMCD, inner medullary collecting duct.
Staining intensity: −, no staining; ±, equivocal positive staining; +, weak positive staining; ++, moderate positive staining; +++, strong positive staining.

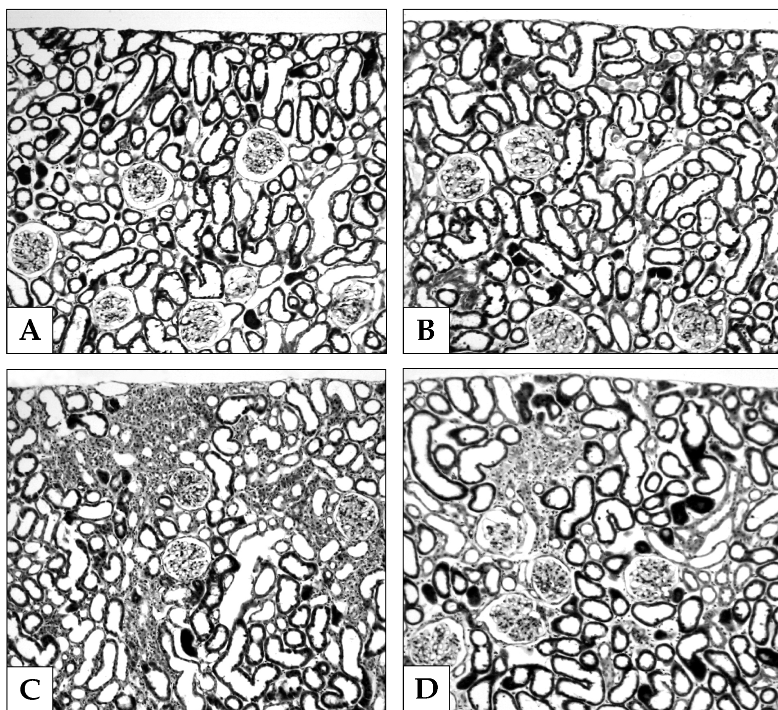

Fig. 1. Effect of RGTZ treatment on interstitial fibrosis in chronic CsA nephropathy. There was no significant interstitial fibrosis in the VH group (A) or VH + RGTZ group (B). However, CsA treatment for 4 weeks induced typical striped interstitial fibrosis, tubular atrophy, and inflammatory cell infiltration in the cortex, as assessed by trichrome staining (C). Note that RGTZ treatment significantly decreased tubulointerstitial fibrosis in the chronic CsA nephropathy (D). Magnification: $\times$ 200.

Immunohistochemistry

The intensity of PPARγ immunoreactivity in the glomeruli and renal tubules in the four groups is summarized in Table 2. In the VH-treated rat kidneys, PPARγ protein was constitutively expressed in the cortex and medulla (Fig. 2). In the cortex, PPARγ immunoreactivity was mainly observed in the glomerular epithelial cells, distal convoluted tubule cells (DT), and collecting tubule cells (CCD). In the medulla, PPARγ immunoreactivity was seen in the inner medullary collecting tubules (IMCD). The CsA group had lower PPARγ immunoreactivity than the VH group (Fig. 3). In the distal convoluted tubules, PPARγ immunoreactivity was almost undetectable, and the cortical collecting tubules had weak PPARγ immunoreactivity compared with the VH group. Exogenous administration of RGTZ increased the overall immunoreactivity of PPARγ in both the VH and CsA groups (Fig. 3). In the VH + RGTZ group, PPARγ immunoreactivity in the distal convoluted tubules and cortical collecting ducts was markedly increased compared with the VH group (Fig. 3B1-B2), and PPARγ expression was also observed in proximal tubular cells. In the CsA + RGTZ group, overall PPARγ immunoreactivity was greater than in the CsA group, and both distal convoluted tubules and cortical collecting tubules showed increased PPARγ immunoreactivity compared with the CsA group.

Western blot analysis

PPARγ immunoblotting and relative optical densities are shown in Fig. 4. Western blotting for PPARγ in the cortex revealed a single band at 55 kDa (Fig. 4A). PPARγ protein was lower in the CsA-treated rat kidneys compared with the VH group (47 $\pm$ 6% vs. 100 $\pm$ 18%, $p < 0.05$). Administration of RGTZ to VH-treated rats increased PPARγ expression by 3.4-fold compared with the VH-treated rat kidney. A similar effect of RGTZ on PPARγ expression was observed in CsA-treated rat kidneys. Exogenous administration of RGTZ significantly increased PPARγ expression compared with the CsA group (223 $\pm$ 31% vs. 47 $\pm$ 6%. $p < 0.05$).

RT-PCR

The influence of CsA-induced renal injury on PPARγ mRNA expression was assessed by RT-PCR analysis. Figure 4B shows the ratios of

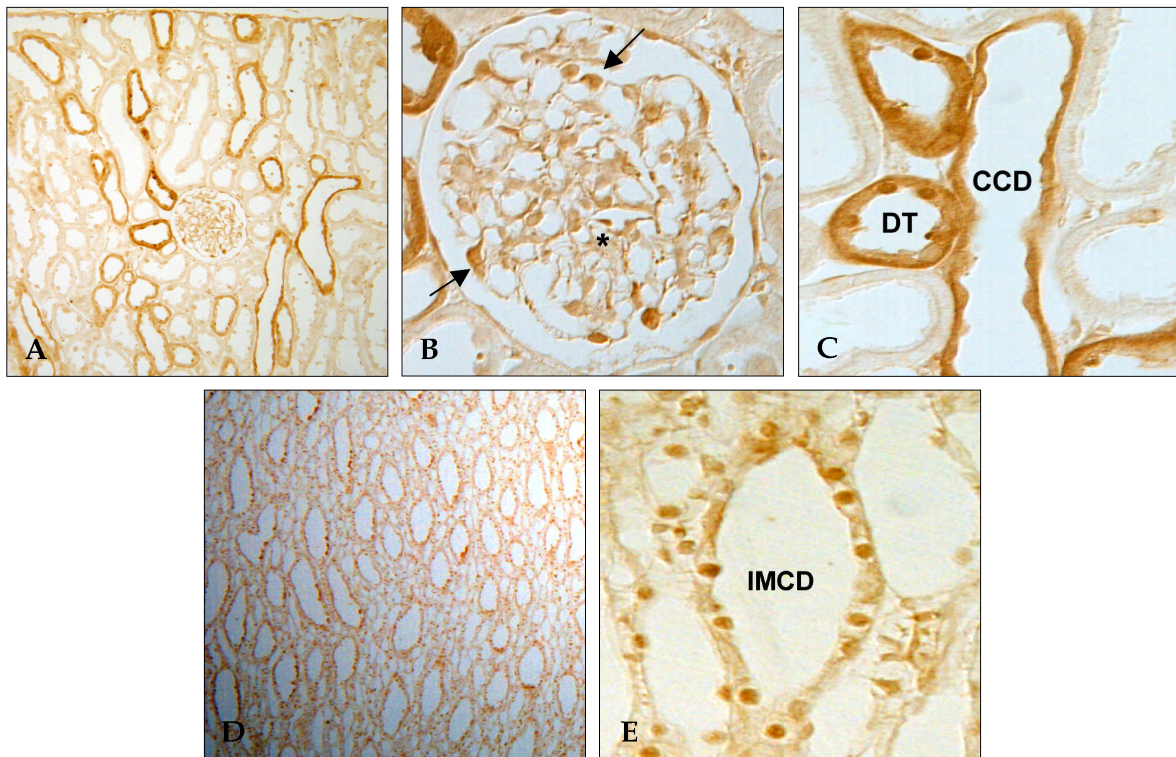

Fig. 2. Representative photomicrographs of immunohistochemistry of PPARγ in VH-treated rat kidney. (A-C) PPARγ immunoreactivity in the cortex. PPARγ protein was mainly located in the glomerular podocytes, distal convoluted tubules and collecting ducts. (D, E) PPARγ immunoreactivity in the medulla. PPARγ protein was mainly located in the inner medullary collecting tubules. Original magnification: × 100.

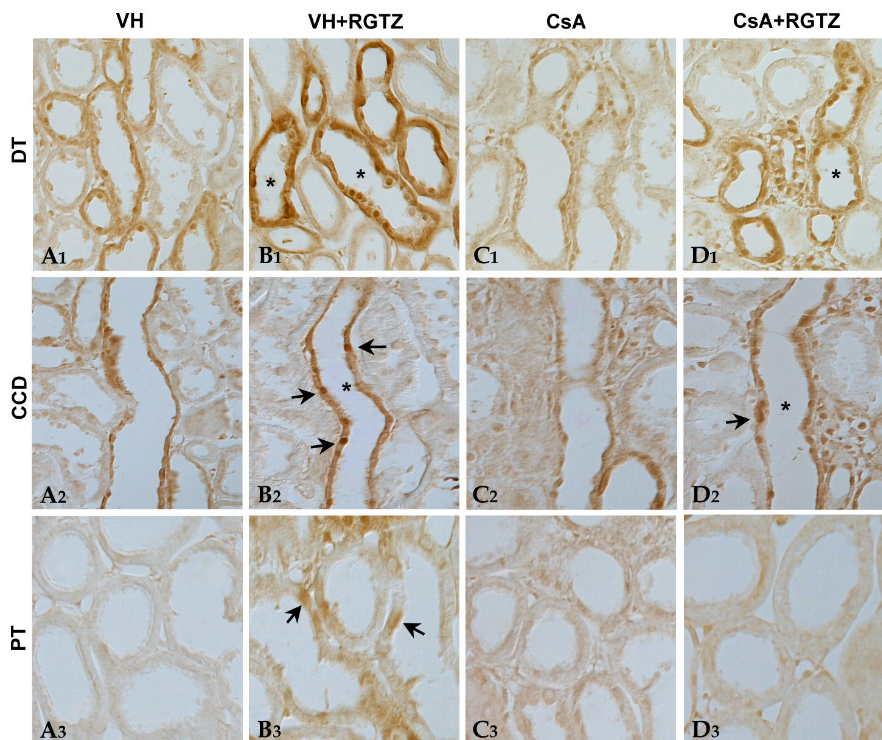

Fig. 3. Comparison of PPARγ protein immunoreactivity in the experimental groups. Note the more intense PPARγ immunoreactivity in the VH + RGTZ groups (B1-B3) compared with the VH group (A1-3), and the increased PPARγ immunoreactivity in the CsA + RGTZ group (D1-D3) compared with the CsA group (C1-C3). Magnification: × 400.

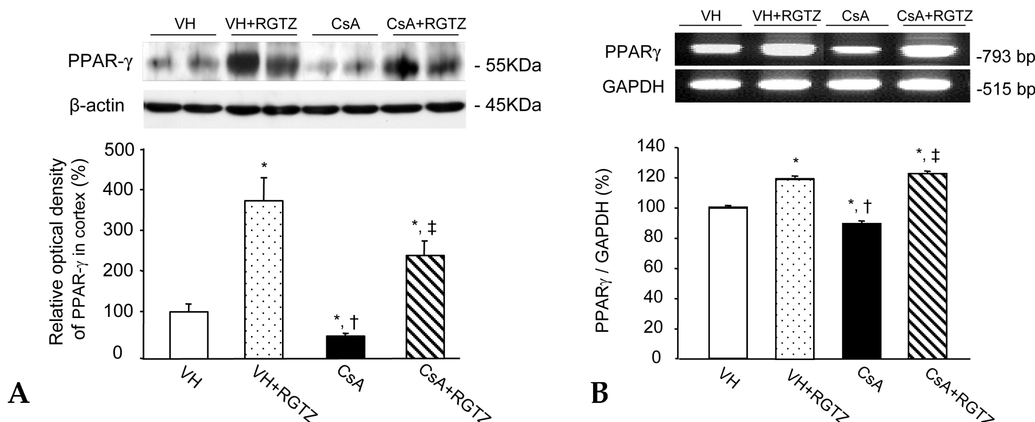

Fig. 4. Immunoblotting and RT-PCR of PPARγ in the experimental groups. (A) Immunoblotting of PPARγ protein. CsA treatment significantly reduced PPARγ protein expression compared with VH alone, but concomitant treatment of RGTZ significantly increased PPARγ protein expression over VH or CsA alone. The expression of PPARγ protein was determined with reference to β-actin expression. (B) RT-PCR of PPARγ mRNA. Note a single band of 793bp corresponding to PPARγ. The expression of PPARγ mRNA in the CsA group was significantly lower than in the VH group. The relative optical densities (%) are presented with the VH group designated as 100%. $^*p < 0.05$ vs. VH. $^\dagger p <$ 0.05 vs. VH + RGTZ. $^\ddagger p < 0.05$ vs. CsA.

RT-PCR products of PPARγ mRNA transcripts and glyceraldehydes phosphate dehydrogenase (GAPDH) transcripts. The expression of PPARγ mRNA in the CsA group was significantly lower than in the VH group (89 ± 2% vs. 100 ± 1%, $p < 0.05$). On the other hand, concomitant treatment of RGTZ significantly increased PPARγ mRNA expression compared with CsA treatment alone (122 ± 2% vs. 89 ± 2%, $p < 0.05$).

DISCUSSION

We found that long-term CsA treatment decreased PPARγ mRNA and protein constitutively expressed on renal tubular cells in normal rats. However, concomitant administration of RGTZ restored PPARγ protein expression in the kidneys of the CsA-treated rats. These results suggest that exogenous administration of RGTZ treatment upregulates PPARγ expression and that this may play a role in protecting against CsA-induced renal injury.

The notable finding in this study is that RGTZ treatment induces PPARγ mRNA and protein expression in the rat kidney. In VH-treated rat kidney, RGTZ treatment caused a 3.4-fold increase of PPARγ protein expression over the VH group without RGTZ treatment. Immunohistochemical analysis revealed that RGTZ treatment further increased immunoreactivity of PPARγ on distal tubules compared with the VH group. Furthermore, RGTZ treatment newly developed PPARγ on proximal tubules (Fig. 3B$_3$). In CsA-treated rat kidney, concomitant treatment of RGTZ caused a 4.7-fold increase of PPARγ protein and a 2.2-fold increase over the VH group. A similar effect of RGTZ on PPARγ mRNA expression was observed in the VH and CsA groups. These findings suggest that RGTZ induces PPARγ mRNA and protein in normal and CsA-treated rat kidneys. Induction of PPARγ on proximal tubules by RTGZ may be associated with the renoprotective effect of RGTZ against CsA-induced renal injury, since the proximal tubules are the main target site of CsA-induced renal injury.[15-20]

The mechanism by which RGTZ treatment upregulates PPARγ mRNA and protein is still undetermined. We suggest two possibilities. The first is that the induction of PPARγ mRNA and protein by RGTZ may inhibit the action of angiotensin II in CsA-induced renal injury.[21] The second possibility is that RGTZ-induced PPARγ mRNA and protein may protect against CsA-induced renal injury by inhibiting thromboxan receptor expression and thromboxan synthase

expression.[22,23] We have recently demonstrated that PPARγ activation leads to the inhibition of thromboxane receptor expression in vascular smooth muscle cells[22] and thromboxane synthase expression in macrophages.[24] Therefore, inhibition of the thromboxane system by activated PPARγ may protect against CsA-induced renal injury.

In general, treatment of PPARγ agonists causes systemic fluid retention, and edema is one of the limitations of PPARγ agonists in clinical practice. Recent reports have revealed that treating cultured collecting ducts with thiazolidinediones increased amiloride-sensitive Na(+) absorption and Scnn1g mRNA (encoding the epithelial Na(+) channel ENaCγ) expression through a PPARγ-dependent pathway.[25] This finding was confirmed with an *in vivo* study demonstrating that weight gain was blocked by the collecting duct-specific diuretic amiloride or by deletion of PPARγ from the collecting ducts. In this study, we also expected weight gain in RGTZ-treated rats, but there was no significant difference in body weight between the VH and VH + RGTZ groups. This finding may have been due to the fact that PPARγ expression in the inner medulla was not greater with RGTZ treatment (Table 2), indicating that sodium reabsorption may not increase in these tubules after treatment. In addition, the low salt diet (0.05% NaCl) used in this study may have prevented edema formation.

In conclusion, RGTZ, a PPARγ agonist, induces PPARγ mRNA and protein expression in the kidney, which may provide the rationale for PPARγ agonist administration for the prevention of CsA-induced renal injury.

REFERENCES

1. Bennett WM, DeMattos A, Meyer MM, Andoh T, Barry JM. Chronic cyclosporine nephropathy: the Achilles' heel of immunosuppressive therapy. Kidney Int 1996; 50:1089-100.
2. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. N Engl J Med 1984;311:699-705.
3. Campistol JM, Sacks SH. Mechanisms of nephrotoxicity. Transplantation 2000;69 (12 Suppl):S5-10.
4. Pichler RH, Franceschini N, Young BA, Hugo C, Andoh TF, Burdmann EA, et al. Pathogenesis of cyclosporine nephropathy: roles of angiotensin II and osteopontin. J Am Soc Nephrol 1995;6:1186-96.
5. Guan Y, Breyer MD. Peroxisome proliferator-activated receptors (PPARs): novel therapeutic targets in renal disease. Kidney Int 2001;60:14-30.
6. Sato K, Sugawara A, Kudo M, Uruno A, Ito S, Takeuchi K. Expression of peroxisome proliferator-activated receptor isoform proteins in the rat kidney. Hypertens Res 2004;27:417-25.
7. Buckingham RE, Al-Barazanji KA, Toseland CD, Slaughter M, Connor SC, West A, et al. Peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, protects against nephropathy and pancreatic islet abnormalities in Zucker fatty rats. Diabetes 1998; 47:1326-34.
8. Yue Tl TL, Chen J, Bao W, Narayanan PK, Bril A, Jiang W, et al. *In vivo* myocardial protection from ischemia/reperfusion injury by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. Circulation 2001;104:2588-94.
9. Wayman NS, Hattori Y, McDonald MC, Mota-Filipe H, Cuzzocrea S, Pisano B, et al. Ligands of the peroxisome proliferator-activated receptors (PPAR-gamma and PPAR-alpha) reduce myocardial infarct size. FASEB J 2002;161:1027-40.
10. Chung BH, Li C, Sun BK, Lim SW, Ahn KO, Yang JH, et al. Rosiglitazone protects against cyclosporine-induced pancreatic and renal injury in rats. Am J Transplant 2005;5:1856-67.
11. Sugawara A, Yen PM, Qi Y, Lechan RM, Chin WW. Isoform-specific retinoid-X receptor (RXR) antibodies detect differential expression of RXR proteins in the pituitary gland. Endocrinology 1995;136:1766-74.
12. Lim SW, Li C, Sun BK, Han KH, Kim WY, Oh YW, et al. Long-term treatment with cyclosporine decreases aquaporins and urea transporters in the rat kidney. Am J Physiol Renal Physiol 2004;287:F139-51.
13. Yang CW, Ahn HJ, Kim WY, Li C, Jung JY, Yoon SA, et al. Synergistic effects of mycophenolate mofetil and losartan in a model of chronic cyclosporine nephropathy. Transplantation 2003;75:309-15.
14. Yang T, Michele DE, Park J, Smart AM, Lin Z, Brosius FC 3rd, et al. Expression of peroxisomal proliferator-activated receptors and retinoid X receptors in the kidney. Am J Physiol 1999;277:F966-73.
15. Healy E, Dempsey M, Lally C, Ryan MP. Apoptosis and necrosis: mechanisms of cell death induced by cyclosporine A in a renal proximal tubular cell line. Kidney Int 1998;54:1955-66.
16. Kim SI, Song HY, Hwang JH, Chong DL, Lee HY, Han DS, et al. Cyclosporine nephrotoxicity: the mechanisms of cell injury by cyclosporine A in renal proximal tubular cells. Transplant Proc 2000;32:1621-2.
17. Hortelano S, Castilla M, Torres AM, Tejedor A, Bosca L. Potentiation by nitric oxide of cyclosporin A and FK506-induced apoptosis in renal proximal tubule cells. J Am Soc Nephrol 2000;11:2315-23.
18. Slattery C, Campbell E, McMorrow T, Ryan MP.

Cyclosporine A-induced renal fibrosis: a role for epithelial-mesenchymal transition. Am J Pathol 2005; 167:395-407.
19. McMorrow T, Gaffney MM, Slattery C, Campbell E, Ryan MP. Cyclosporine A induced epithelial-mesenchymal transition in human renal proximal tubular epithelial cells. Nephrol Dial Transplant 2005; 20:2215-25.
20. Bone JM, Amara AB, Shenkin A, Hammad A, Sells RA, Alexander JL, et al. Calcineurin inhibitors and proximal renal tubular injury in renal transplant patients with proteinuria and chronic allograft nephropathy. Transplantation 2005;79:119-22.
21. Yang CW, Ahn HJ, Kim WY, Shin MJ, Kim SK, Park JH, et al. Influence of the renin-angiotensin system on epidermal growth factor expression in normal and cyclosporine-treated rat kidney. Kidney Int 2001;60: 847-57.
22. Sugawara A, Uruno A, Kudo M, Ikeda Y, Sato K, Taniyama Y, et al. Transcription suppression of thromboxane receptor gene by peroxisome proliferator-activated receptor-gamma via an interaction with Sp1 in vascular smooth muscle cells. J Biol Chem 2002;277: 9676-83.
23. Darlametsos IE, Papanikolaou EN, Varonos DD. Effect of nifedipine in cyclosporine-induced nephrotoxicity in rats: roles of the thromboxane and endothelin systems. Prostaglandins Leukot Essent Fatty Acids 2000;63:263-9.
24. Darlametsos IE, Varonos DD. Role of prostanoids and endothelins in the prevention of cyclosporine-induced nephrotoxicity. Prostaglandins Leukot Essent Fatty Acids 2001;64:231-9.
25. Guan Y, Hao C, Cha DR, Rao R, Lu W, Kohan DE, et al. Thiazolidinediones expand body fluid volume through PPARgamma stimulation of ENaC-mediated renal salt absorption. Nat Med 2005;11:861-6.