Protective effect of peroxisome proliferator-activated receptor-gamma agonists on activated renal proximal tubular epithelial cells in IgA nephropathy

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Abstract

Background. We have previously demonstrated a glomerulo-tubular ‘crosstalk’ operating in the pathogenesis of tubulointerstitial injury in IgA nephropathy (IgAN). The present study aims to explore any possible beneficial effect of a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist in alleviating the tubulointerstitial inflammation in IgAN.

Methods. Human proximal tubular epithelial cells (PTEC) were pre-treated with increasing concentration of a PPAR-γ agonist rosiglitazone or troglitazone (0–5 µM) followed by further incubation with the conditioned medium (IgA-HMC) collected from human mesangial cells (HMC) incubated with polymeric IgA isolated from IgAN patients. Gene expression of interleukin-6 (IL-6) and angiotensin II type 1 receptor (ATR1) was determined by ELISA and western blot, respectively. The mitogen-activated protein kinase extracellular signal-related kinase 1/2 (ERK1/2) activation was examined by western blot.

Results. An IgA-HMC conditioned medium prepared from IgAN patients increased gene expression and protein synthesis of IL-6 and ATR1 in PTEC when compared with a conditioned medium prepared from healthy controls. The unregulated expression and protein synthesis of IL-6 and ATR1 in PTEC induced by the IgA-HMC conditioned medium were readily attenuated following pre-treatment with a PPAR-γ agonist, thiazolidinedione (TZD). The ATR1-downregulating effect exerted by the PPAR-γ agonist occurred through the inhibition of ERK1/2 activation. The PPAR-γ antagonist, GW9662, significantly attenuated the inhibitory action of rosiglitazone on the increased synthesis of IL-6 and ATR1 protein.

Conclusion. Our current findings suggest that the PPAR-γ agonist attenuates excessive inflammatory response in activated PTEC in IgAN through suppressing ATR1 expression. This ATR1-downregulating effect is likely through the inhibition of ERK1/2 activation and is found to be PPAR-γ dependent. TZDs may possibly be new therapeutic additives to established treatment regime for renal–angiotensin system (RAS) blockade in IgAN.
Keywords: angiotensin II type 1 receptor; IgA nephropathy; PPAR-gamma agonist; tubulointerstitial injury; tubular epithelial cells

Introduction

IgA nephropathy (IgAN), the most common type of primary glomerular disease, is an immune complex-mediated glomerulonephritis with predominant mesangial IgA deposition accompanied by a variety of renal lesions [1]. The deposited IgA, mainly polymeric IgA1, triggers inflammatory cascades within the kidney including the proliferation of mesangial cells, extracellular matrix formation, glomerular and tubular inflammation that finally lead to renal fibrosis [2]. Eventually, ~40% of patients develop end-stage renal disease (ESRD) and this progression is closely correlated with tubulointerstitial damage where IgA deposition is barely detected [3]. Our recent studies reveal that renin–angiotensin system (RAS) activation is pivotal in the pathogenesis of tubulointerstitial injury in IgAN [4]. Blockade of RAS using either the angiotensin-converting enzyme (ACE) inhibitor (ACEI) or AngII type 1 receptor (ATR1) antagonist (ARB) remains the first-line choice for treating IgAN with the unavailability of clearance of mesangial IgA deposition and the extreme variability of disease presentation and clinical course [5]. However, the full clinical efficacy is still limited by the maximal dose of ACEI or ARB [4]. Hence, to explore other alternative means to suppress the intrarenal RAS effectively in IgAN is of great significance.

Peroxisome proliferator-activated receptors-γ (PPAR-γ) are a member of the nuclear hormone receptor family with three identified isoforms and is differentially expressed in several tissues including the kidney. Isoforms 1 and 3 are identical and only differ in their splice variants when fully translated. PPAR-γ2 is predominantly found in adipocytes and differs from the other isoforms at its extended N-terminus [6]. By binding to the peroxisome proliferator response element (PPRE) in target genes, PPAR-γ regulates many cellular processes such as glucose metabolism and adipogenesis [6]. Apart from its glucose-lowering potency in diabetes, PPAR-γ activation has also been verified to exert anti-inflammatory and anti-fibrotic effect in kidney diseases [7]. More interestingly, the activation of PPAR-γ suppresses ATR1 gene expression and thus inhibits AngII-induced cell growth and hypertrophy in vascular smooth muscle cells (VSMC) [8,9].

Herein, we hypothesize that PPAR-γ activation also suppresses ATR1 expression in the kidney, and hence blocks the local activation of RAS and attenuates the excessive inflammatory response mediated by ATR1 in IgAN. We first examined tubular expression of PPAR-γ isoforms before studying the effect of two PPAR-γ agonists on inflammatory responses in activated tubular cells.

Materials and methods

Reagents

 Dulbecco’s modified Eagle’s and Ham’s F12 media (DMEM/F12 medium), fetal bovine serum (FBS), antibiotics, sera, agaro and DNA size markers were purchased from Invitrogen Co. (Carlsbad, CA, USA). Reagents for CDNA synthesis were obtained from Promega (Paisley, UK) and those for polymerase chain reaction (PCR) were from Perkin Elmer (Branchburg, NJ, USA). The enzyme immunooassay kit for detection of interleukin-6 (IL-6) was purchased from Bender MedSystems (Vienna, Austria). The ATR1 antibody and actin antibody were from Lab Vision Co. (Fremont, USA). The mitogen-activated protein kinase extracellular signal-related kinase 1/2 (ERK1/2) antibody was obtained from Cell Signalling Technology (Beverly, CA, USA). Anti-mouse and anti-rabbit secondary antibodies were from Dako A/S (Glostrup, Denmark). Lactate dehydrogenase (LDH) release and bromodeoxyuridine (BrdU) incorporation assay kits were from Roche Diagnostic (Indianapolis, IN, USA). The synthetic PPAR-γ agonist rosiglitazone and troglitazone were obtained from GlaxoSmithKline (Middlesex, UK) and Sigma (St Louis, MO, USA).

Patients and control subjects

Thirty Chinese patients (18 male and 12 female) with clinical and renal immunopathologic diagnosis of primary IgAN were studied. IgAN was diagnosed by the presence of predominant granular IgA deposits, mainly in the glomerular mesangium and occasionally along the peripheral capillary basement membrane by immunofluorescence examination, and the presence of mesangial electron-dense deposits in ultrastructural examination. All the patients were symptomatic for >12 months, and no significant renal impairment was documented. Systemic lupus erythematosus, Henoch-Schönlein purpura (HSP) and hepatic diseases were excluded by detailed clinical history, examination and negative laboratory findings for hypocomplementaemia, anti-DNA antibody or hepatitis B virus surface antigen. Twenty milliliters of blood was collected from each patient at clinical quiescence. The serum was isolated and frozen at −20°C until isolation of IgA by Jacalin–agarose affinity column.

Thirty healthy subjects (18 male and 12 female), comparable in age, sex and race, with no microscopic haematuria or proteinuria, were recruited as normal control subjects. Serum was similarly collected from these individuals for processing. Informed written consent for blood sampling was obtained from every subject. The study was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the institutional ethics committees.

Preparation of IgA

Polymeric IgA1 (MW > 320 kDa) was isolated and purified from sera of patients with IgAN or controls as previously described [4]. Analysis of the glycosylation profile revealed that the anionic fraction of these pIgA1 preparation was underglycosylated with higher content of α(2,6)-linked sialic acid [10].

Cell culture

Human mesangial cells (HMC) and proximal tubular epithelial cells (PTEC) were isolated as previously described [11]. Renal cortical tissues were obtained from kidneys that were removed for circumcised tumours. Histological examination of these kidney samples revealed no renal pathology. Cortical specimens were cut into small cubes and passed through a series of mesh sieves of a diminishing pore size. Briefly, PTEC were collected on the 53 µm sieve and digested with collagenase (750 U/ml) at 37°C for 15 min. Tubular cells were isolated by centrifugation and grown in a 1:1 mixture of DMEM and Ham’s F12 media supplemented with 10% FCS, penicillin (100 IU/ml) and streptomycin (10 µg/ml). The cells were incubated at 37°C in 5% CO2 and 95% air. The cells were characterized by immunofluorescence and immunohistochemistry staining positive for cytokeratin, vimentin and alkaline phosphatase but negative for Tamm-Horsfall glycoprotein, factor VIII-related antigen and α-smooth muscle actin. Scanning electron microscopy demonstrated the presence of numerous apical microvilli of a rudimentary brush border with reassembly of tight junctions. Experiments were performed with cells up to the third passage as preliminary studies showed no phenotypic changes up to this passage number. In all experiments, there was a ‘growth arrest’ period of 24 h in a serum-free medium before stimulation. Results were
obtained from PTEC that were cultured from the kidney of three different donors.

**Preparation of conditioned medium**

Growth-arrested HMC were cultured in six-well culture plates (1 × 10^6 cells per well) with culture medium containing 0.5% FBS and pIgA (final concentration 50 μg/ml) isolated from 30 IgAN patients and 30 paired healthy control subjects for 48 h. The conditioned medium after culture was collected and kept frozen at −70°C until use. The plain medium control was defined as PTEC cultured in the plain medium without any addition of IgA. For dose-dependent experiments, the 30 conditioned media (individual IgA-HMC conditioned medium from IgAN or control) were randomly divided into five groups and were pooled to form five pools (the pooled IgA-HMC conditioned medium with conditioned media from six individual patients or controls in each pool).

**Cytotoxicity and proliferation of PTEC under incubation with PPAR-γ agonists**

Rosiglitazone and troglitazone were dissolved in DMSO. To test whether the concentration of these drugs and the final DMSO concentration used were cytotoxic, growth-arrested PTEC were seeded into 96-well plates (0.25 × 10^5 cells per well) before exposure to rosiglitazone, troglitazone or DMSO (0–0.4%) for 24 h or 48 h. Next, the cytotoxic effect of these thiazolidinediones (TZDs) on PTEC was examined by a LDH release assay. Briefly, a 100 μl reaction mixture (provided by the diagnostic kit) was added to each well to incubate with cells in the dark at room temperature (RT) until colour development. The reaction was terminated with 50 μl stopping reagent (provided by the kit), and the absorbance was measured at 490 nm with the reference at 630 nm by an ELISA reader.

The cell proliferation under the incubation of TZDs was examined by a BrdU incorporation assay. Briefly, after cells were incubated with TZDs or DMSO for 24 h or 48 h in 96-well plates (0.25 × 10^5 cells per well), a 10 μl BrdU labelling solution was added to the wells and incubated for 2 h at 37°C. After this incubation, the labelling medium was removed and 200 μl working solution was added before incubation for further 90 min at RT. The FixDenat solution was then removed, and a 100 μl/well anti-BrdU-POD working solution was added before incubation for further 90 min at RT. After the incubation, the antibody conjugate was removed followed by adding a 100 μl/well substrate solution. The incubation was continued until colour development. The reaction was terminated with 1 M H2SO4, and the absorbance was measured by an ELISA reader at 450 nm with 690 nm as the reference wavelength. All results were expressed as percentage changes in absorbance compared with that of the medium control (defined as PTEC incubated with the plain culture medium).

**Total RNA extraction and reverse transcription (RT)-polymerase chain reaction**

Growth-arrested PTEC were pre-incubated with or without TZDs for 1 h and followed by exposure of an IgA-HMC conditioned medium from IgAN patients for 4 h. Total cellular RNA was extracted using a NucleoSpin RNA II total RNA extraction kit (Macherey-Nagel, Düren, Germany). The quality of the extracted RNA was monitored by formaldehyde agarose gel electrophoresis. Four micrograms of total RNA was reverse-transcribed to cDNA with Moloney Murine Leukemia Virus reverse transcriptase (Promega, Madison, WI, USA) in a 20 μl reaction mixture containing 50 ng random hexamer, 0.5 mmol/l dNTPs and 20 U of an RNase inhibitor. The cDNA was stored at −20°C until further use. Gene expression of PPAR-γ1/3, PPARγ-2, ATR1 and IL-6 by PTEC was examined by PCR using specific primers (Table 1) designed from known sequences in the GenBank. For semi-quantification, human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers (Table 1) were included in every reaction as an internal control. PCR products were analysed by agarose gel electrophoresis and stained with ethidium bromide. Images of the gel were captured using the Gel Doc 1000 gel Documentation Densitometry System from Bio-Rad (Hercules, CA, USA). The product yield was expressed as a ratio to GAPDH.

**ELISA of IL-6 protein synthesis in cell culture supernatants**

Growth-arrested PTEC were pre-incubated with or without TZDs for 1 h and followed by exposure of IgA-HMC conditioned medium from IgAN patients for 48 h. Cells culture supernatants were collected and stored at −70°C until protein assay. The IL-6 protein level in culture supernatants was determined by a commercial assay kit. The detection sensitivity for IL-6 was 1.6 pg/ml while the intra-batch coefficient of variation was 6.3%.

**Western blot analysis of ATR1 and ERK1/2**

After collecting the cell culture supernatant as mentioned above, the remaining cells were lysed with a lysis buffer that contained protease inhibitor cocktails (Sigma, St Louis, MO, USA). The protein concentrations were measured by a modified Lowry method using BSA as standard (DC Protein Assay Kit; Bio-Rad). Ten micrograms of the total protein extracted from 10^6 cells was electrophoresed through a 12% SDS–PAGE gel before being transferred to a polyvinylidene difluoride membrane. After blocking for 1 h at room temperature in a blocking buffer [5% bovine serum albumin in Tris–buffered saline (TBS) with 0.05% Tween-20 (TBST)], the membrane was incubated overnight with rabbit anti-ATR1 (1:1000) or rabbit anti-phosphorylated-ERK1/2 (1:300) in TBST. The membrane was washed and incubated for 1 h at room temperature with a peroxidase-labelled goat anti-rabbit immunoglobulin. After further washing, the membrane was detected with ECL chemiluminescence (Amersham Pharmacia Biotech, Arlington, IL, USA).

**Statistical analysis**

All data were expressed as means ± SD unless otherwise specified. Statistical analysis was performed using SPSS v.15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Intergroup differences for continuous variables were assessed by multivariate ANOVA. P < 0.05 was considered statistically significant.

**Results**

**Activation of PTEC by an IgA-HMC conditioned medium from IgAN patients**

Confluent, growth-arrested cells were exposed either for 4 h (gene expression) or 48 h (protein synthesis) to a plain
medium alone or supplemented with an IgA-HMC conditioned medium collected from IgAN patients or healthy controls at descending fold of dilutions with plain medium (40×–10×). As shown in Figure 1, PTEC were significantly activated by the IgA-HMC conditioned medium from IgAN patients with upregulated gene expression (Figure 1A and B) and protein synthesis (Figure 1C and D) of IL-6 and ATR1 in a dose-dependent manner (P < 0.05, P < 0.01). A 20-fold dilution of the IgA-HMC conditioned medium was chosen for stimulation in later experiments.

Expression of PPAR-γ isoforms by PTEC

We then investigated whether PPAR-γ, the binding target of PPAR-γ agonists, was expressed in PTEC. Using a specific pair of primers to detect the PPAR-γ2 isoform and another pair to detect the common sequence for both PPAR-γ1 and PPAR-γ3 isoforms, we found that PTEC expressed only the PPAR-γ1/3 isoform while the PPAR-γ2 isoform was undetectable by RT–PCR (Figure 2A). White blood cells (WBC) and adipocytes (AC) were used as positive control for PPAR-γ1/3 and PPAR-γ2 isoforms. The gene expression of PPAR-γ was not altered following culture with pIgA from patients with IgAN or controls for HMC as previously reported [12] or for PTEC (data not shown). Incubating PTEC with an IgA-HMC conditioned medium did not alter the gene expression of PPAR-γ (data not shown).

Effect of PPAR-γ agonists on cell cytotoxicity and proliferation in PTEC

Cytotoxicity and proliferation in PTEC following culture with serial dilutions of rosiglitazone, troglitazone or DMSO were tested using LDH release and BrdU incorporation assay, respectively. In cell cytotoxicity tested by LDH release assay, incubation with rosiglitazone up to the highest concentration (40 µM) and with troglitazone up to 20 µM for 24 h and 48 h failed to show any increase in LDH release (Figure 2B and C). In cell proliferation study determined by the BrdU incorporation assay, concentrations of rosiglitazone and troglitazone <5 µM did not affect the cell proliferation (Figure 2D and E). Cells cultured under these corresponding basal concentrations of DMSO did not show any significant decrease in cytotoxicity or proliferation (data no shown). Five micromole was selected as the maximum dosage for both rosiglitazone and troglitazone in our subsequent experiments.

PPAR-γ agonists did not affect IL-6 and ATR1 gene expression in PTEC

In order to dissect the mere effect of PPAR-γ agonists on IL-6 and ATR1 expression in PTEC, we incubated PTEC with increasing concentration of rosiglitazone or troglitazone (0.625–5 µM) for 5 h without other additions and using TNF-α (10 ng/ml) stimulation as the positive control.
Fig. 2. PPAR-γ isoforms gene expression in PTEC and cell cytotoxicity and proliferation of PTEC cultured with PPAR-γ agonists. (A) Compared with positive controls including white blood cells (WBC) and adipocytes (AC) that are known to express both PPAR-γ1/3 and PPAR-γ2 isoforms, cultured PTEC expressed only the PPAR-γ1/3 isoform, not the PPAR-γ2 isoform by RT–PCR detection. LDH release (B, C) and BrdU incorporation (D, E) by PTEC cultured with different concentrations of rosiglitazone or troglitazone (0.625–40 µM) for either 24 h or 48 h. Results are expressed as percentage changes in absorbance relative to that of the PTEC incubated with the plain medium alone. *P < 0.05, **P < 0.01 versus PTEC cultured with the plain medium control. All results represent means ± SD obtained from three independent experiments.

No significant difference was observed between the TZDs treatment and the plain medium control in either IL-6 or ATR1 gene expression (Figure 3A and B, P > 0.05).

PPAR-γ agonists downregulated the overexpression of IL-6 induced by an IgA-HMC conditioned medium in PTEC

To study the dose-dependent effect of PPAR-γ ligation on IL-6 release from PTEC induced by the IgA-HMC conditioned medium, we treated PTEC with rosiglitazone or troglitazone (0.625–5 µM) for 1 h before and during the incubation with a 20-fold diluted pooled IgA-HMC conditioned medium (4 h for gene expression and 48 h for protein synthesis). Rosiglitazone and troglitazone attenuated the upregulation of IL-6 gene expression (Figure 4A and B, P < 0.01) and protein synthesis (Figure 4C and D, P < 0.05) in PTEC induced by a pooled IgA-HMC conditioned medium from IgAN patients in a dose-dependent manner.

We next examined the effect of rosiglitazone pre-incubation (at a concentration of 5 µM) on IL-6 release in PTEC under the stimulation by the individual IgA-HMC conditioned medium (30 IgAN patients and 30 healthy controls). Rosiglitazone significantly inhibited the upregulated IL-6 gene expression (Figure 4E) and protein synthesis (Figure 4F) in PTEC induced by the individual IgA-HMC conditioned medium from IgAN patients (P < 0.01).

PPAR-γ agonists downregulated the overexpression of ATR1 induced by an IgA-HMC conditioned medium in PTEC

Parallel experiments were conducted to study the effect of PPAR-γ agonists on ATR1 expression. Both rosiglitazone and troglitazone attenuated the upregulation of ATR1 gene expression (Figure 5A and B) and protein synthesis (Figure 5C and D) in PTEC induced by a pooled IgA-HMC conditioned medium from IgAN patients in a dose-dependent manner (P < 0.01 and P < 0.05). Similarly, we studied the effect of rosiglitazone (5 µM) on ATR1 expression in PTEC induced by an individual IgA-HMC conditioned medium each prepared separately from 30 individual IgAN patients and 30 individual healthy controls. Rosiglitazone significantly attenuated the ATR1 gene expression (Figure 5E) and protein synthesis in PTEC (Figure 5F) induced by the individual IgA-HMC conditioned medium from IgAN patients (P < 0.01).
Rosiglitazone attenuated activation of the ERK1/2 pathway induced by an IgA-HMC conditioned medium in PTEC

To explore whether ATR1-associated signalling pathway was also attenuated by the PPAR-γ agonist, PTEC were treated with rosiglitazone (5 µM) 1 h before and during 48 h incubation with an IgA-HMC conditioned medium. The cell lysates were collected for measuring ERK1/2 phosphorylation by western blotting. As shown in Figure 6A, compared with plain medium control and pooled IgA-HMC conditioned medium from healthy controls (PC), the pooled IgA-HMC conditioned medium from IgAN patients (PA) significantly increased the phosphorylation of ERK1/2 in PTEC (P < 0.01). Rosiglitazone preincubation reduced the ERK1/2 signalling pathway activation induced by the IgA-HMC conditioned medium from IgAN patients in PTEC (P < 0.01).

PPAR-γ-dependent effect of rosiglitazone in PTEC activated by an IgA-HMC conditioned medium

PPAR-γ ligands possess both PPAR-γ-dependent and independent effects. In order to determine whether the downregulatory effect of rosiglitazone was PPAR-γ dependent or not, we performed parallel experiments in PTEC.
adding GW9662 (25 μM), a specific PPAR-γ antagonist, 30 min before the 1 h incubation of rosiglitazone (5 μM) followed by 48 h stimulation with an IgA-HMC conditioned medium. GW9662 significantly reversed the inhibitory effect of rosiglitazone on enhanced ATR1 (Figure 6B) and IL-6 (Figure 6C) protein synthesis in PTEC induced by the IgA-HMC conditioned medium from IgAN patients (P < 0.05).

Discussion

Following our recent findings demonstrating glomerulopodocytic and glomerulo-tubular ‘crosstalks’ operating in IgAN [11,13], we further confirmed in this study that mesangial IgA deposition in IgAN leads to release of soluble mediators, mainly TNF-α and AngII, which could then activate renal TEC by increasing
their IL-6 synthesis. The IL-6, in turn, increases the ATR1 expression and gradually enhances AngII production in PTEC forming a vicious circle that leads to excessive inflammatory response in tubular cells [11].

Accumulating evidences have also identified the detrimental role of intrarenal RAS in the disease progression of IgAN, in which AngII is the key effector mediating through ATR1 [4]. To block the activation of the RAS either by ACEI, ARB or the combination is the mainstay
Treatment with PPAR-γ agonists in IgAN

Fig. 6. Inhibitory effect on ERK1/2 signalling pathway and PPAR-γ-dependent effect on IL-6 and ATR1 protein synthesis of rosiglitazone in PTEC cultured with a pooled IgA-HMC conditioned medium. Western blot analysis (A) of phosphorylated ERK1/2 demonstrated that rosiglitazone (5 μM) incubation 1 h before and during the 48 h stimulation of a pooled IgA-HMC conditioned medium significantly abolished the activation of ERK1/2 in PTEC induced by an IgA-HMC conditioned medium from IgAN patients. PPAR-γ antagonist GW9662 (25 μM) significantly attenuated the inhibitory effect of rosiglitazone on IL-6 (B), ATR1 (C) protein over-synthesis induced by an IgA-HMC conditioned medium from IgAN patients. All results represent means ± SD obtained from three independent experiments. Representative images of western blot band showing a phosphorylated ERK1/2 protein are shown at the top of each panel.

of treatment for IgAN [5,14]. However, even at effective anti-hypertensive doses, the tissue concentration of either compound is still far lower than that of serum and is less likely to suppress the local intrarenal RAS adequately [4]. Hence, alternative therapeutic option targeting at the tubular RAS is highly desirable as tubulointerstitial injury bears a prognostic index in IgAN.

PPAR-γ belongs to the family of nuclear hormone receptors and is a ligand-activated transcription factor with at least three isoforms been identified, PPAR-γ1, PPAR-γ2 and PPAR-γ3, producing two types of proteins PPAR-γ1 and PPAR-γ2. Its protective effect on kidney diseases has been well illustrated [7]. In vitro, PPAR-γ suppresses inflammatory response and proliferation of mesangial cells [15,16], reduces secretion of fibronectin [17] and type collagen [18] by mesangial cells. In cultured TEC, it also facilitates their recovery under the stimulation of high glucose [19], hydrogen peroxide [20] and glycated albumin [21] and limits pro-inflammatory and fibrotic responses when exposed to albumin [22]. In vivo, PPAR-γ agonists, TZDs, attenuate nephropathy in murine models of type 2 diabetes [23] and non-diabetic glomerulosclerosis [24] and decrease proteinuria patients with type 2 diabetes [25] and non-diabetic renal disease [26]. However, data on the renoprotective effect of PPAR-γ on IgAN are still lacking.

We first examined the expression of PPAR-γ isoforms in the renal interstitium as limited published studies so far revealed controversial findings. A discrepancy between the localization of PPAR-γ mRNA and protein expression has been reported that a predominant expression of PPAR-γ1 protein is detected in the cortex while gene expression of PPAR-γ1 is more abundant in the medulla than in the cortex [27,28]. As for PPAR-γ2, neither gene nor protein expression is detected in the kidney [27]. In line with previous findings, we also only demonstrated the gene expression of the PPAR-γ1/3 isoform but not the PPAR-γ2 isoform in cultured PTEC. We then applied two PPAR-γ agonists, rosiglitazone and troglitazone, to our in vitro cell culture model and found that both agonists significantly inhibited the overexpression of IL-6 induced by the IgA-HMC conditioned medium from IgAN patients. The attenuation of inflammatory responses in activated PTEC clearly supported an anti-inflammatory effect of PPAR-γ in IgAN. With the absence of PPAR-γ2 in renal tissues, it is indicated that, in the present study, the anti-inflammatory effect of either rosiglitazone or troglitazone (being agonists for both PPAR-γ1 and PPAR-γ2 [29]) is mediated through the activation of PPAR-γ1 in cultured PTEC.

Interestingly, PPAR-γ activation has also been shown to reduce ATR1 transcription in VSMC [8,9]. Hence, we further explored whether the anti-inflammatory effect of PPAR-γ agonists in IgAN may also be mediated through reduction of ATR1 expression in TEC. We measured both gene expression and protein synthesis of ATR1 in PTEC
pre-incubated with PPAR-γ agonists followed by the culture with the IgA-HMC conditioned medium. Upregulated ATR1 expression in PTEC induced by the IgA-HMC conditioned medium collected from IgAN patients was significantly attenuated by both rosiglitazone and troglitazone, further extending the therapeutic potential of PPAR-γ activation in the disease progression by down-regulating the ATR1 expression in renal TEC. The mechanism by which the ATR1 expression is down-regulated was first elaborated by Sugawara et al. [8] who showed that PPAR-γ activation suppressed the ATR1 gene at a transcriptional level by inhibiting Sp1 promoter elements via a protein–protein interaction. This pathophysiological mechanism was later confirmed in an animal model [30] and proven to be PPAR-γ dependent [31]. In addition, other signalling pathways may also be operative. PPAR-γ activation has been shown to inhibit the activation of inflammatory response genes by negatively interfering with the transcription factors nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT) and activator protein-1 (AP-1) in macrophages [32]. It has been shown that NF-κB and AP-1 can induce ATR1 gene transcription [33,34], and are under the regulation of the ERK1/2 signal pathway. ERK1/2, at the same time, is the downstream pathway of AngII-mediated tubular inflammation via ATR1 [35]. Therefore, a positive feedback operates between ERK1/2 and ATR1, leading to excessive inflammatory responses in the disease state, including IgAN [36]. Herein, in our in vitro model of IgAN, we examined the effect of the PPAR-γ agonist on ERK1/2 activation and found that rosiglitazone significantly abolished ERK1/2 activation in PTEC activated by the IgA-HMC conditioned medium. In this circumstance, we speculate that PPAR-γ may also down-regulate ATR1 transcription in PTEC through suppressing ERK1/2 signalling pathway and finally attenuate the excessive inflammatory responses induced by the IgA-HMC conditioned medium from IgAN patients. Alternatively, a recent study demonstrated that PPAR-γ possessed anti-inflammatory properties not only at transcriptional level but also at level of upstream receptor signalling through suppression of receptor-mediated reactive oxygen species generation [37]. Further studies are warranted to specifically dissect the mechanism of PPAR-γ activation in the treatment of IgAN.

The receptor-dependent and independent effect of PPAR-γ agonists is another issue of interest [38]. In order to define the action of the PPAR-γ agonist rosiglitazone, we used a PPAR-γ antagonist, GW9662, to test the receptor-dependent effect. We found that GW9662 fully attenuated the inhibitory effect of rosiglitazone on the enhanced synthesis of IL-6 and ATR1 protein indicating that the anti-inflammatory effect of rosiglitazone was, at least, partially PPAR-γ dependent.

**Conclusion**

Our current findings suggest that the PPAR-γ agonist, in a PPAR-γ-dependent manner, attenuates excessive inflammatory response in activated PTEC in IgAN through suppressing ATR1 expression. This ATR1-downregulating effect is likely through the inhibition of ERK1/2 activation. Thiazolidinediones may possibly be new therapeutic additives to ACEI and ARB in achieving a better RAS blockade for treating IgAN.

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**Conflict of interest statement.** None declared.

**References**

Efficacy of the combination of N-acetylcysteine and desferrioxamine in the prevention and treatment of gentamicin-induced acute renal failure in male Wistar rats*

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Abstract

Background. Oxidative stress and the formation of aminoglycoside–iron complexes through iron-dependent Fenton reaction have been proposed to be the major mechanisms in the development of GM-induced acute renal failure (ARF); however, the efficacy of the combination of N-acetylcysteine (NAC) and desferrioxamine (DFX) in the prevention and the treatment of GM-induced ARF has not previously been investigated.

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