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PPAR-α and -γ agonists attenuate diabetic kidney disease in the apolipoprotein E knockout mouse

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Abstract

Backgound. Peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ agonists are widely used in diabetes. In addition to their effects on lipid and glucose homeostasis, these agents have been postulated to have independent renoprotective actions. In the current study, we assess the efficacy of the PPAR-α agonist, gemfibrozil, the PPAR-γ agonist rosiglitazone and the non-thiazolidinedione PPAR-α/γ coagonist, compound 3q, on kidney structure and function in streptozotocin-treated apolipoprotein E knockout mice.

Methods. Control and streptozotocin-diabetic mice were randomized to receive rosiglitazone (20 mg/kg/day), gemfibrozil (100 mg/kg/day), or compound 3q (3 mg/kg/day) by gavage, or no treatment for a period of 20 weeks. Renal fibrosis was assessed by standard histology and collagen IV immunohistochemistry. Kidney function was assessed by urinary albumin excretion and creatinine clearance.

Results. Diabetes in this model was associated with an increase in glomerulosclerosis, tubulointerstitial fibrosis and increased collagen IV deposition in the glomeruli and tubules. All three agents significantly attenuated glomerulosclerosis, tubulointerstitial expansion and collagen IV deposition. The increase in albuminuria and the decline in kidney function associated with diabetes in this model were also attenuated by each of these agents, with no superiority observed among various treatment groups. These renoprotective effects were observed in the absence of changes in glucose, insulin or lipid levels or a reduction in blood pressure.

Conclusions. Combined with their independent anti-atherosclerotic actions, and their important effects on dyslipidaemia and insulin resistance, PPAR agonists may be useful for the prevention of diabetic complications, including kidney disease, even in type 1 diabetes.

Keywords: diabetes; diabetic kidney disease; glomerulosclerosis; insulin-dependent diabetes mellitus; peroxisome proliferator-activated receptor; tubulointerstitial fibrosis

Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear transcription factors that have been shown to play important roles in maintaining glucose and lipid homeostasis. The PPAR-α agonists, such as gemfibrozil and fenofibrate, are utilized in the management of dyslipidaemia, increasing high-density lipoprotein (HDL) cholesterol levels and reducing triglyceride and, to a lesser extent, low-density lipoprotein (LDL) cholesterol. The PPAR-γ agonists, such as rosiglitazone and pioglitazone, are agents that improve glycaemic control by increasing insulin sensitivity, predominantly through effects on free fatty acid metabolism in adipose tissue and skeletal muscle. In individuals with type 2 diabetes, both PPAR-α and PPAR-γ agonists have particular utility, given the important role of dyslipidaemia and insulin sensitivity, respectively, in the development and progression of diabetic complications.

PPAR agonists may also have beneficial actions in diabetes, over and above their effects on metabolic control. For example, we have recently published elsewhere that PPAR agonists mediate direct anti-atherogenic effects in the diabetic vasculature, independent of their metabolic actions [1,2]. PPAR agonists also have independent anti-inflammatory and anti-proliferative actions, which may contribute to their clinical efficacy, particularly in terms of end-organ injury. There has been only limited research into the direct effects of PPAR agonists on the progression of diabetic kidney disease. In some studies, PPAR-γ

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agonists have been associated with a reduction in the markers of fibrosis and hypertrophy in models of experimental diabetes [3–5]. However, the independent effects of PPAR-α agonists in diabetic kidney disease have not been previously investigated. In addition, despite the imminent clinical advent of dual PPAR-α/γ agonists, the potential interaction of PPAR-α and PPAR-γ agonists in this process has not been defined.

This study aims to examine the direct effects of PPAR-α, PPAR-γ and PPAR-α/γ agonists, independent of their actions on insulin sensitivity and dyslipidaemia, on kidney structure and function in the insulin-deficient diabetic apolipoprotein E knockout (apoE-KO) mouse.

Methods

Animals

Six-week-old male apoE-KO mice (backcrossed 20 times to a C57BL/6 background; Animal Resource Centre, Canning Vale, WA, Australia and maintained in the Precinct Animal Centre, Melbourne, Australia) were used in this study. The apoE gene deletion in mice results in chronic hypercholesterolaemia that is not responsive to treatment with PPAR-α agonists or hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors [6]. Although best known as a model of aortic plaque formation, the diabetic apoE-KO mouse is also an established model of progressive renal injury [6,7]. The protocols for animal experimentation and the handling of animals were in accordance with the principles established by the Animal Welfare Committee of the Baker Institute/Alfred Hospital and National Health and Medical Research Council ethical guidelines.

Experimental diabetes model

apoE-KO mice were randomized for the induction of insulin deficient diabetes via intraperitoneal injection of streptozotocin (55 mg/kg; MP Biomedicals, Eschwege, Germany) daily for 5 days. This model was chosen to eliminate potentially confounding effects of improved metabolic control associated with improved insulin sensitivity following the use of PPAR agonists. Animals with more than 10% glycated haemoglobin 10 weeks after the induction of diabetes, were included in the study as diabetic. Sham-injected control animals (sodium citrate buffer pH 4.5) were followed concurrently. Diabetic and control animals were further randomized to receive the PPAR-γ agonist, rosiglitazone (20 mg/kg/day), the PPAR-α agonist, gemfibrozil (100 mg/kg/day) or the non-thiazolidinedione PPAR-α/γ co-agonist, (S)-3-(4-(2-carbazol-9-yl-ethoxy)phenyl)-2-ethoxy-propionic acid (compound 3q) (3 mg/kg/day) by gavage, or no treatment for a period of 20 weeks ($n = 12$/group). None of the animals with diabetes required supplemental insulin to maintain body weight or prevent ketosis. Throughout the study, mice were given access to standard chow and water *ad libitum*.

After 20 weeks of experimental diabetes, mice were culled via intraperitoneal injection of Euthal (10 mg/kg; Delvet Limited, Seven Hills, Australia) followed by exsanguination by cardiac puncture. The kidneys were rapidly dissected out, weighed and processed to paraffin for subsequent analysis.

Measurement of physiological and biochemical parameters

Before sacrifice, mice were placed in individual metabolic cages (Iffa Credo, L'Arbresele, France) for a period of 24 h. Body weight, food and water intake were recorded. Urine was collected for subsequent analysis (see further). Blood glucose was measured serially using a glucometer (Accutrend; Boehringer Mannheim GmbH. Biochemica, Mannheim, Germany). Glycated haemoglobin (Hb) was measured in whole blood obtained at the time of sacrifice, by high performance liquid chromatography (HPCL; CLC330 GHb Analyzer; Primus, Kansas City, MO, USA.). Plasma glucose, total cholesterol, LDL, HDL and triglycerides were measured in samples obtained at the time of sacrifice, using an automated system (Abbott Architect ci8200, Abbott Laboratories, IL). In addition, fasting serum insulin concentration was measured by radioimmunoassay (Linco Research, St Charles, MI).

Estimation of systolic blood pressure

Systolic blood pressure (mmHg) was assessed in conscious mice using the computerized non-invasive tail-cuff method. Mice were familiarized with the equipment to ensure accurate measurements and readings were taken by an experienced technician on conscious mice at the conclusion of the study.

Quantitation of kidney fibrosis

To evaluate kidney histopathology, 3 μm kidney sections were stained with periodic acid–schiff (PAS). Glomerulosclerotic injury (GSI) was graded based on the severity of glomerular damage, including mesangial matrix expansion, hyalinosis with focal adhesion, capillary dilation, glomerular tuft occlusion and sclerosis [7]. Specifically, grade 0 represents intact glomerulus; grade 1 represents <25% glomerular injury; grade 2 represents 25–50% glomerular injury; grade 3 represents 50–75% glomerular injury and grade 4 represents 75–100% injury. Twenty glomeruli were assessed per kidney in a masked fashion. GSI was calculated using the formula:

$$\mathrm{GSI} = \frac{(1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)}{(n_0 + n_1 + n_2 + n_3 + n_4)}$$

where n_x = number of glomeruli in each grade of injury.

Tubulointerstitial area (TIA) was estimated at the corticomedullary junction using a point counting system. For each field, 100 points were assessed on a 1 cm^2 eyepiece graticule with 10 equidistant gridlines. Six high-power fields (400×) were analysed per kidney. Results are expressed as the percentage of tubulointerstitial space within the area assessed.

Diabetic kidney disease in the apoE-KO mouse has previously been shown to be associated with an increased accumulation of extracellular matrix proteins in the mesangium and tubulointerstitial compartments [7]. As an additional measure of kidney fibrosis in this study, protein expression of matrix protein, collagen IV was also assessed in these two compartments. Sections were rehydrated, washed and then incubated in 3% H_2O_2 (v/v) for 20 min to quench endogenous hydrogen peroxide. To facilitate antigen retrieval, slides were pre-digested with 0.4% pepsin

(Sigma-Aldrich, St Louis, MO) in 0.01N hydrochloric acid for 10 min at 37°C. After washing, the slides were incubated in 0.5% milk for 15 min. The primary antibody to collagen IV (Southern Biotechnology, Birmingham, AL) diluted 1/1000 in 1% horse serum in tri-buffered saline (TBS) was then added to sections and left overnight at 4°C. Avidin and biotin binding were then specifically blocked and secondary antibody, anti-goat immunoglobulin (Vector Laboratories, Burlingame, CA) was added at a dilution of 1:500 for 10 min at room temperature. Avidin-biotin complex reagent (ABC) was then added for 30 min at room temperature (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA). Peroxidase conjugates were visualized with 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St Louis, MO). Images were quantitated on an Olympus BX50 microscope using Optimas (v6.2) and digitized using a colour video camera (3-CCD; JVC, Wayne, NJ).

Measurement of kidney function

The urinary albumin excretion rate (AER) was measured by radioimmunoassay, using a rabbit anti-mouse albumin antibody (Dako Corporation, Carpinteria, CA), as established in our laboratory [7]. Urine and serum creatinine were measured by HPLC according to the animal models of diabetic complications consortium (AMDCC) guidelines. Briefly, aliquots of urine and plasma were mixed with acetyl nitrile (sample: 1:5), centrifuged at 4°C. The supernatant was then removed, dried in a speedivac, then resuspended in 10 mM ammonium acetate, pH 3.2. Samples were then injected into a C_{18} column (Waters Division of Millipore, Marlborough, MA, USA) and detected at 235 nm using a Hewlett Packard PDA detector. Creatinine clearance was estimated as the ratio of daily urinary creatinine excretion to plasma creatinine concentration, and expressed as millilitre per minute.

Results

Metabolic parameters and blood pressure

Experimental diabetes was associated with a greater than 3-fold increase in plasma glucose levels. Similarly, there was a significant increase in glycated haemoglobin in diabetic animals. Treatment with rosiglitazone, gemfibrozil and compound 3q marginally improved the mean glycaemic control, in both control and diabetic animals (Table 1). However, none of these treatments normalized the glycated haemoglobin levels in diabetic animals, or had any significant effect on fasting plasma glucose levels.

Treatment with streptozotocin was associated with a significant fall in fasting insulin levels in the presence of chronic hyperglycaemia, consistent with the induction of insulinopaenic diabetes. Treatment with rosiglitazone, gemfibrozil and compound 3q had no effect on plasma insulin concentrations in diabetic mice, or on weight loss associated with diabetes. However, in the control mice, all three treatments resulted in improved insulin sensitivity, associated with reduced plasma insulin levels (Table 1).

The induction of diabetes was also associated with a significant increase in the total and LDL cholesterol concentration compared with control animals (C *vs* D, $P < 0.001$). None of the three treatments had any significant effect on this parameter in diabetic mice. Plasma triglyceride concentrations were unaffected by the induction of diabetes. As previously reported in control apoE-KO mouse, LDL cholesterol was reduced by gemfibrozil [1]. Treatment with compound 3q was associated with a significant reduction in HDL cholesterol levels, and an increase in triglyceride concentrations in diabetic animals (Table 1). The induction of experimental diabetes did not result in any significant change in the systolic blood pressure levels. Treatment with rosiglitazone, gemfibrozil or compound 3q did not reduce the systolic blood pressure in diabetic mice (Table 1).

Kidney hypertrophy

All animals with diabetes developed kidney hypertrophy, as indicated by increased kidney/body weight ratio (Table 2). Treatment with the PPAR-γ agonists, rosiglitazone and compound 3q significantly reduced the kidney hypertrophy associated with diabetes, although not to control levels. Gemfibrozil had no effect on kidney/body weight ratio. Notably, compound 3q also reduced the kidney/body weight ratio in the control mice (Table 2).

Table 1. Metabolic parameters at the conclusion of the study

Parameter	C	C + R	C + G	C + 3q	D	D + R	D + G	D + 3q
Body weight (g)	30.0 ± 0.6	28.5 ± 0.5	28.8 ± 0.6	29.6 ± 0.5	23.4 ± 0.5*	22.8 ± 0.5	22.7 ± 0.9*	23.8 ± 0.8*
Glucose (mmol/l)	9.4 ± 1.4	12.2 ± 1.4	10.5 ± 1.1	11.2 ± 0.6	35.0 ± 1.4*	34.2 ± 1.5*	35.1 ± 1.8*	36.5 ± 1.2*
Glycated Hb (%)	4.8 ± 0.1	3.7 ± 0.0*	3.5 ± 0.1*	3.8 ± 0.0*	17.1 ± 0.3*	15.9 ± 0.3*†	15.5 ± 0.7*†	13.8 ± 0.7*†
Insulin (ng/ml)	0.47 ± 0.06	0.31 ± 0.05*	0.32 ± 0.13*	0.24 ± 0.08*	0.18 ± 0.05*	0.11 ± 0.02*	0.12 ± 0.03*	0.19 ± 0.06*
Triglycerides (mmol/l)	1.2 ± 0.2	1.9 ± 0.5	1.0 ± 0.5	3.2 ± 0.7*	1.5 ± 0.1	1.7 ± 0.2*	1.5 ± 0.2	1.7 ± 0.1*
Total cholesterol (mmol/l)	12.3 ± 0.7	16.3 ± 2.1	8.8 ± 1.4	8.1 ± 1.8*	24.6 ± 0.9*	28.2 ± 1.5*	26.8 ± 1.7*	26.2 ± 5.8*
LDL cholesterol (mmol/l)	8.9 ± 0.6	8.6 ± 0.5	3.6 ± 0.5*	7.1 ± 1.4	13.9 ± 1.4*	18.0 ± 1.8*	15.7 ± 1.4*	14.1 ± 2.7*
HDL cholesterol (mmol/l)	3.1 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	1.1 ± 0.2*	3.6 ± 0.3	3.8 ± 0.3	3.2 ± 0.5	1.9 ± 0.4†*
Systolic BP (mmHg)	105 ± 1	103 ± 1	102 ± 1	106 ± 1	101 ± 1	106 ± 2†	108 ± 2†	108 ± 2†

Glycated haemoglobin (GHb), blood pressure (BP). Data shown as mean ± SEM. *$P < 0.05$ compared with control mice; †$P < 0.05$ compared with diabetic mice. Control (C), control + rosiglitazone (C + R), control + gemfibrozil (C + G), control + compound 3q (C + 3q), diabetic (D), diabetic + rosiglitazone (D + R), diabetic + gemfibrozil (D + G), diabetic + compound 3q (D + 3q).

Table 2. Markers of renal function at the conclusion of the study

Parameter	C	C + R	C + G	C + 3q	D	D + R	D + G	D + 3q
Kidney/body weight ratio	12.4 ± 0.1	11.8 ± 0.2	12.0 ± 0.2	11.2 ± 0.2*	16.4 ± 0.3*	15.6 ± 0.3**	16.8 ± 0.4	14.7 ± 0.3**
Creatinine clearance (ml/min)	0.19 ± 0.01	0.15 ± 0.01*	0.19 ± 0.01	0.19 ± 0.01	0.14 ± 0.01*	0.15 ± 0.01*	0.19 ± 0.01**	0.19 ± 0.01**
Urinary AER (μg/day)	5.0 ± 0.6	8.2 ± 0.8	8.4 ± 0.7	4.5 ± 0.6	52.9 ± 3.6*	41.6 ± 3.0***	43.1 ± 2.6***	39.1 ± 3.6***
Glomerulosclerosis (%)	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.8 ± 0.1*	2.6 ± 0.1**	2.5 ± 0.1**	2.5 ± 0.1**
Tubulointerstitial area (%)	16.3 ± 0.7	16.4 ± 0.4	17.6 ± 0.7	18.5 ± 0.6*	22.5 ± 0.4*	16.7 ± 0.3**	15.9 ± 0.5**	16.5 ± 0.5**

Data shown as mean ± SEM as fold induction. Albumin excretion rate (AER). *$P < 0.05$ compared with control mice; **$P < 0.05$ compared with diabetic mice.
Control (C), control + rosiglitazone (C + R), control + gemfibrozil (C + G), control + compound 3q (C + 3q), diabetic (D), diabetic + rosiglitazone (D + R), diabetic + gemfibrozil (D + G), diabetic + compound 3q (D + 3q).

Glomerulosclerosis

The induction of diabetes was associated with a significant increase in glomerulosclerosis, characterized by the accumulation of extracellular matrix protein in the mesangium (Figure 1 and Table 2). Treatment with rosiglitazone, gemfibrozil or compound 3q, all resulted in a significant reduction in glomerulosclerosis, when compared with mice with untreated diabetes. Neither agent appeared to have greater efficacy than the other. Notably, the dual PPAR-α/γ coagonist, compound 3q was not more effective than comparable doses of the PPAR-α or γ agonists. Untreated non-diabetic apoE-KO mice also showed significant glomerulosclerosis, consistent with the previously described effect of chronic dyslipidaemia on kidney morphology in this model [6,7]. However, the treatment with rosiglitazone, gemfibrozil or compound 3q had no effect on this parameter in non-diabetic apoE-KO animals.

Tubulointerstitial expansion

The induction of diabetes was associated with a significant increase in TIA, associated with an increased infiltration of mononuclear cells, dilation of tubules and tubulointerstitial matrix deposition (Figure 1 and Table 2). As seen with respect to glomerulosclerosis, treatment with rosiglitazone, gemfibrozil or compound 3q resulted in a significant reduction in tubulointerstitial expansion associated with diabetes. Of the three agents, gemfibrozil appeared to be the most effective, although this difference was not significant. None of the treatments were able to reduce TIA in non-diabetic apoE-KO animals.

Matrix protein deposition

The induction of diabetes was associated with increased immunostaining for collagen IV in both the glomerular and tubular compartments (Figures 1 and 2). Treatment with rosiglitazone, gemfibrozil or compound 3q, all resulted in a significant reduction in tubulointerstitial collagen IV deposition associated with diabetes. However, only rosiglitazone and gemfibrozil were effective at reducing glomerular collagen IV staining in diabetic mice. These agents were also effective in reducing collagen IV deposition in non-diabetic apoE-KO animals.

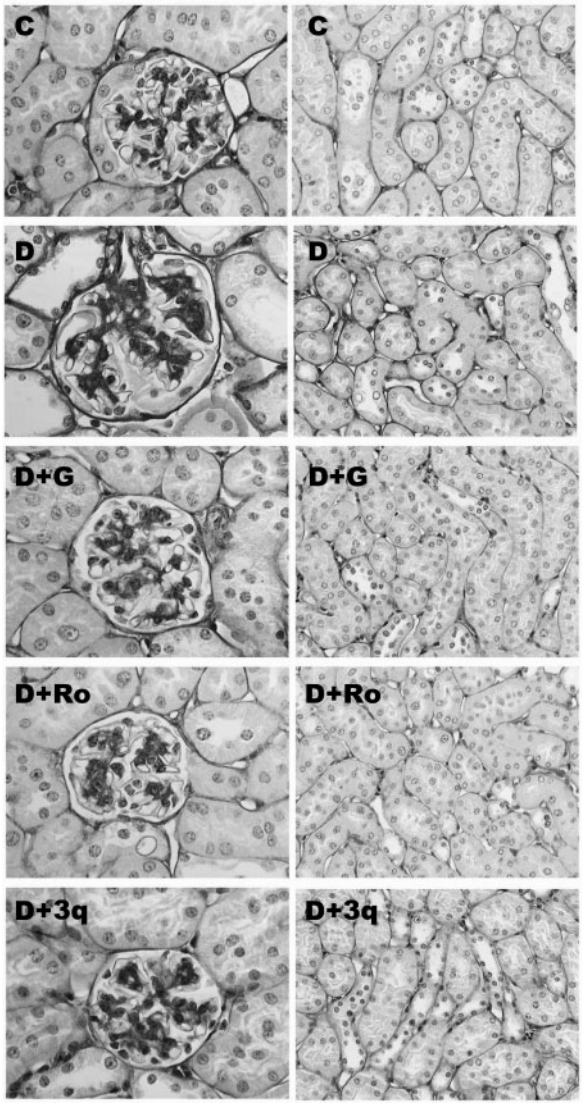

Fig. 1. Representative pictures of glomerular (left panel) and tubular staining (right panel) for collagen IV for control (C), diabetic (D), diabetic + rosiglitazone (D + R), and diabetic + gemfibrozil (D + G), and diabetic + compound 3q (D + 3q) mice. Magnification (800×).

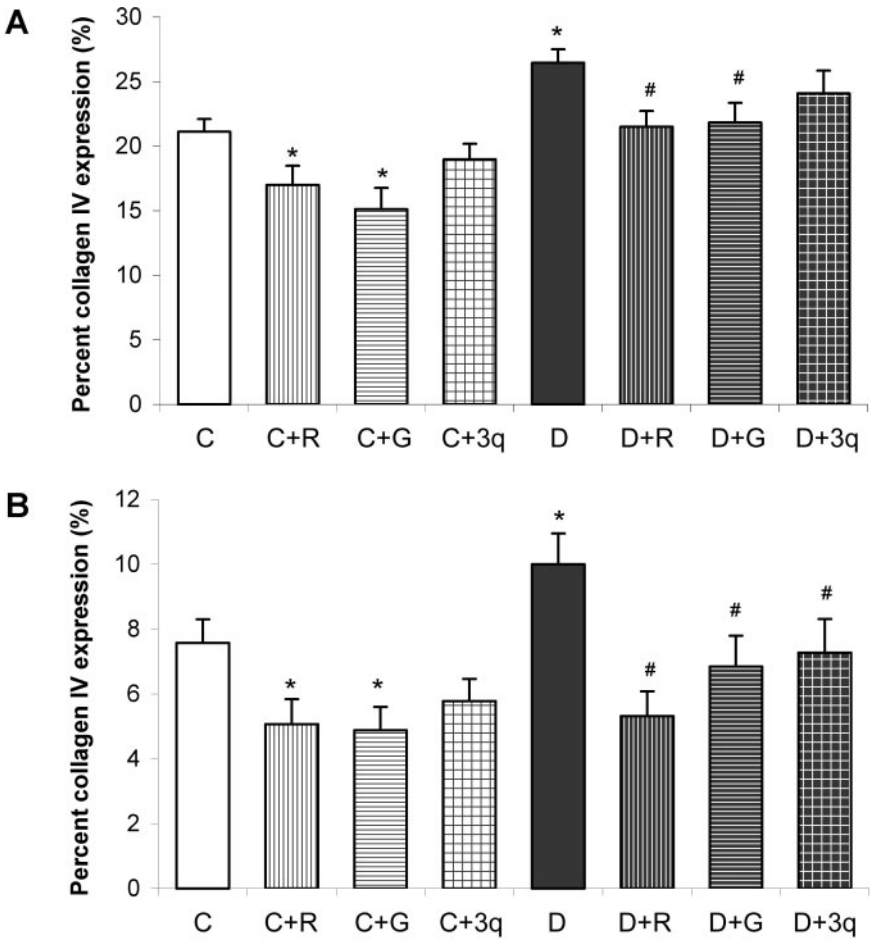

Fig. 2. Quantification of glomerular (**A**) and tubular (**B**) staining for collagen IV in the renal cortex of study groups. Control (C), control + rosiglitazone (C + R), control + gemfibrozil (C + G), control + compound 3q (C + 3q), diabetic (D), diabetic + rosiglitazone (D + R), diabetic + gemfibrozil (D + G), and diabetic + compound 3q (D + 3q) mice.

Urinary albumin excretion

As previously described in this model [7], the induction of diabetes was associated with a 5-fold increase in urinary albumin excretion, when compared with non-diabetic apoE-KO mice. Consistent with the effects observed on kidney structural parameters, treatment with rosiglitazone, gemfibrozil or compound 3q, all resulted in a significant reduction in urinary albumin excretion associated with diabetes (Table 2), although urinary albumin excretion remained elevated when compared with non-diabetic animals. These agents had no effect on urinary albumin excretion in non-diabetic apoE-KO mice.

Creatinine clearance

Untreated diabetes was also associated with significantly reduced kidney function compared with apoE-KO mice (Table 2). Treatment with gemfibrozil or compound 3q resulted in significant improvements in kidney function, returning creatinine clearance to control levels (Table 2). Treatment with rosiglitazone resulted in a significant reduction in creatinine clearance in control animals, consistent with previous reports [8]. However, the induction of diabetes in animals treated with rosiglitazone did not result in any additional impairment of kidney function.

Discussion

PPAR agonists have a number of pleiotropic effects, independent of their actions on metabolic control that may confer a particular utility for these agents in the prevention of diabetic kidney disease. In this study, we demonstrate that the PPAR-α agonist, gemfibrozil, the PPAR-γ agonist rosiglitazone and the non-thiazolidinedione PPAR-α/γ co-agonist, compound 3q, have renoprotective actions in experimental diabetes, in the absence of effects on plasma glucose, blood pressure or lipid levels. By utilizing a model of insulinopaenic diabetes in our study, we aimed to examine the effects of PPAR-γ activation, independent of the effects on insulin signalling, on the development and progression of diabetic kidney disease. Similarly, by utilizing the apoE-KO mouse model of

dyslipidaemia that is not responsive to PPAR-α agonists, we aimed to differentiate the direct actions of these agents from those occurring as a result of actions on circulating lipid levels.

PPAR-α agonists are widely used in patients with diabetes. Their beneficial effects have largely been attributed to the modulation of dyslipidaemia [9–11]. In our study we show that, independent of lipid lowering, the PPAR-α agonist, gemfibrozil is able to reduce kidney hypertrophy and fibrosis associated with diabetic kidney disease. These findings are consistent with results from the Diabetes Atherosclerosis Interventional Study [12], where fenofibrate reduced albuminuria independent of the changes in lipid parameters. PPAR-α ligands have also been shown to have renoprotective actions in other models of progressive renal injury [13,14]. Moreover, PPAR-α KO mice have increased kidney/bodyweight ratios and evidence of cortical damage [15]. However, this is the first description of *in vivo* utility of a PPAR-α agonist in diabetic kidney disease.

PPAR-γ agonists have a number of metabolic effects that potentially influence the outcomes of diabetic kidney disease, including improvements in glycaemic control, insulin sensitivity and dyslipidaemia. These actions appear to be superior to that of other anti-diabetic drugs [16–18]. The renoprotective activity of PPAR-γ agonists in our study is consistent with previous studies showing that thiazolidinediones are able to attenuate kidney disease in streptozotocin diabetes [4,5], Zucker rats [3] and in patients with diabetes [16–18]. Findings in other models of progressive renal damage also suggest that PPAR-γ agonists have independent renoprotective actions [19].

The mechanisms of these renoprotective actions of PPAR-γ agonists remain poorly understood. PPAR-γ agonist ligands can attenuate the expression or activity of NADPH oxidase, an enzyme now considered to be a key pathogenic mediator of injury in the diabetic kidney. Pioglitazone is able to increase the tubular uptake of albumin *in vitro* and at the same time reduces tubular cell production of the inflammatory and profibrotic cytokines and chemokines, such as monocyte chemoattractant protein-1 and transforming growth factor-β1 (TGF-β1) [20]. However, no reduction in the gene expression of TGF-β1, or its key mediator connective tissue growth factor (CTGF), was observed in our model (data not shown). Collagen deposition associated with diabetes may also be attenuated due to direct effects on the modulators of matrix degradation [21]. Notably, this effect appears to be independent of nuclear factor-κB (NF-κB) activity.

The perceived independent actions of PPAR-α and γ agonists have led to the development of dual agonists with glucose and lipid-modulating activity. In this study, we used the non-thiazolindine compound, compound 3q, an agent shown to have full PPAR-α and γ agonistic properties *in vivo*. Previous studies with compound 3q have demonstrated normalization of glucose levels and HbA_{1c} and a reduction of plasma triglycerides to a greater extent than seen with rosiglitazone alone [22]. In our study, compound 3q had also significantly more PPAR-α activity than the pure PPAR-α agonist, gemfibrozil (data not shown). Despite these attributes, we did not demonstrate a superior effect of the dual agonist on any of the standard indices of kidney injury when compared with monotherapy. However, the actions of PPAR-α and PPAR-γ ligands were not equivalent in this model. Only the PPAR-γ agonists were able to attenuate renal hypertrophy. Similarly, only the PPAR-α agonists were able prevent a reduction in creatinine clearance in diabetic animals. However, this may reflect the known negative impact of rosiglitazone on creatinine clearance [8].

Finally, the equivalent effects of PPAR-α and PPAR-γ agonists, as well as thiazolidinedione and non-thiazolidinedione compounds, raise the possibility that these agents also partly act through PPAR-independent pathways in this model. It is known, for example, that some of the actions of fibrates persist in PPAR-α-deficient mice [23]. Similarly, some of the inhibitory effects on gene regulation by thiazolidinediones may not require the presence of the receptor [24,25]. For example, thiazolidinediones are able to inhibit elevated endothelial cell PAI-1, via pathways not involving the activation of the PPAR-γ receptor [25]. The expression of vascular endothelial growth factor is also induced by PPAR agonists via PPAR-dependent and independent mechanisms [26]. PPAR agonists are also able to directly antagonize the activities of other transcription factors including activator protein-1, signal transducers and activators of transcription-1 and NF-κB, possibly by trans-repression of other nuclear receptors [24,27]. These PPAR-independent actions may be considered complementary to the renoprotective effect of PPAR receptor activation, particularly in the diabetic kidney.

In summary, the PPAR-α agonist, gemfibrozil, the PPAR-γ agonist, rosiglitazone and the PPAR-α/γ co-agonist, compound 3q, have equivalent renoprotective actions in experimental diabetes, over and above effects on plasma glucose, blood pressure or lipid levels. This finding is consistent with the important role of the PPAR signalling system in diabetic complications. Moreover, these benefits correlate with the direct anti-atherogenic effects of PPAR agonists observed in the diabetic vasculature [1,2]. The clinical relevance of this finding remains to be established, given the negative effects of the dual agonist, muraglitazar in patients with diabetes [28] and equivocal outcomes and side effects observed in the recent FIELD [29] and proACTIVE [30] studies.

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Conflict of interest statement. None declared.

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