Are peroxisome proliferator-activated receptors new therapeutic targets in diabetic and non-diabetic nephropathies?

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Introduction

Peroxisome proliferator-activated receptors (PPAR), members of the nuclear hormone-receptor superfamily of ligand-binding-transcription factors, are involved in the pathophysiology of the metabolic syndrome. Agonist activation of PPAR provides a new pharmacological pathway to the treatment of the metabolic syndrome and its complications. Since one of the major complications of this syndrome is nephropathy, the potential benefit of PPAR α, -γ and -β/δ agonists on kidney merits examination. Moreover, numerous studies have demonstrated that, in addition to their hypolipidaemic and anti-diabetic effects, these drugs possess anti-inflammatory, anti-fibrotic and anti-proliferative properties. These data strongly suggest a potential benefit of PPAR agonists on diabetic and non-diabetic nephropathies. Herein, we describe the currently known effects of PPAR α, -γ and -β/δ agonists on diabetic and non-diabetic nephropathies, and more precisely, focus on their potential positive impact on kidneys.

Renal effects of PPAR α agonists

PPAR α agonists are involved in lipid-metabolism regulation primarily by increasing fatty acid β oxidation. PPAR α are predominantly expressed in tissues with high peroxisomal β oxidation and mitochondrial activities (liver, heart, skeletal muscle, intestine and kidney) and, to a lesser extent, in other tissues. Pharmacological PPAR α agonists, such as fibrates, are also involved in almost all steps of lipoprotein metabolism. They lower hepatic triglyceride production by increasing fatty acid β oxidation, increase the high-density lipoprotein (HDL)-cholesterol level by raising expression of apolipoproteins A1 and AII, favour reverse cholesterol transfer by increasing hepatic scavenger-receptor class B type 1 (SR-B1), necessary for the uptake of HDL-cholesterol and promote HDL-mediated cholesterol efflux from macrophages located in the vascular wall by inducing ATP-binding
In the kidney, PPARα agonists also lower the triglyceride level by increasing lipoprotein lipase activity which induces lipolysis [1]. In addition to their lipid regulation, PPARα agonists have also been shown to have anti-inflammatory properties. In the kidney, PPARα are predominantly expressed in proximal tubules, the medullary thick ascending limb and, to a lesser degree, in mesangial cells [2].

**Importance of the PPARα-induced β-oxidation pathway for maintaining adequate renal proximal tubule function**

During sustained starvation, PPAR activation plays a crucial role by triggering fatty acid β oxidation to maintain balanced energy production and expenditure. This phenomenon was well-illustrated by Kamijo et al. [3] who showed that sustained starvation of PPARα knockout mice increased urinary albumin excretion (UAE) because of the inability to correctly resorb albumin in renal proximal tubules. This resorption dysfunction is histologically documented by the observation of giant lysosomes containing large amounts of albumin. This failure of lysosomal enzymes to degrade albumin can be reversed by glucose administration, which restores appropriate ATP production. These observations suggest that β-oxidation activation by PPARα agonists is essential for adequate proximal tubule function during sustained starvation.

**PPARα activation prevents excess renal lipid accumulation and renal function deterioration in diabetic and non-diabetic animal models**

Excess lipid storage in the proximal tubules favours renal dysfunction. It most commonly results from on the one hand, enhanced expression of two major transcription factors—sterol regulatory element-binding protein-1 (SREBP-1) and SREBP-2—which stimulate fatty acid and cholesterol syntheses, on the other hand decreased expression of PPARα-dependent key enzymes involved in β oxidation.

In this way, several diabetic animal models with nephropathy such as Zucker diabetic fatty (ZDF) rats, db/db mice [4], SREBP-1c transgenic mice [5], age-related renal disease rat model [6] and high-fat-diet-induced obesity in C57BL/6J mice [7] are associated with excess renal lipid accumulation. This lipid accumulation, depending on the models, may induce an increased production of transforming growth factor-β (TGFβ), vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF), plasminogen-activator inhibitor-1 (PAI-1) and extracellular matrix proteins (type IV collagen and fibronectin) all of which contribute to the resulting glomerular hypertrophy, glomerulosclerosis, tubulointerstitial fibrosis, proteinuria and eventually accelerated deterioration of renal function.

In non-diabetic animal, the models of renal injury, such as ischaemia–reperfusion and cisplatin-induced acute renal failure, are also characterized by excess lipid build-up in renal tubule cells secondary to fatty acid β-oxidation impairment via PPARα-activation pathways [8–10]. The increased amount of cellular lipids, in cisplatin-treated proximal tubule cells in culture, responsible for lipotoxicity, leading to apoptotic cell death, is notably prevented by prior administration of a PPARα agonist [8]. PPARα-null mice, with induced renal ischaemia–reperfusion disease, develop more severe deterioration of renal function than wild type controls [9]. Finally, prior administration of PPARα agonists, in the cisplatin-induced mouse model of renal failure and the Wistar rat model of renal ischaemia–reperfusion injury, significantly protected renal function, primarily by increasing renal fatty acid β oxidation [9,10]. In contrast, the nephroprotective impact of PPARα agonists was not observed when they were administered to PPARα-null mice [9,10].

**Anti-inflammatory properties of PPARα agonists**

PPARα agonists are also nephroprotective because of anti-inflammatory properties. In addition to renal excess lipids accumulation, cisplatin-induced acute renal failure is associated with increased levels of nuclear factor-κB (NF-κB)-binding activity, chemokines, pro-inflammatory cytokines and enhanced neutrophil infiltration into the corticomedullary area of kidney. Prior administration of a PPARα ligand led to significantly fewer inflammatory events caused by cisplatin [11]. In contrast, this anti-inflammatory effect of PPARα agonists in the cisplatin model was not observed in PPARα-null mice.

In another rat model of renal inflammation, characterized by crescentic glomerulonephritis induced by rabbit anti-glomerular basement membrane (GBM) antibodies, high doses of the PPARα agonist bezafibrate significantly prevented glomerular proliferation, macrophage infiltration and proteinuria [12].

**PPARα–cytochrome P-450 activation pathway and pressure natriuresis regulation**

Cytochrome P-450 is a target for PPARα agonists. Its activation induces production of 20-HETE (hydroxy-eicosatetraenoic acid) an arachidonic acid metabolite [13]. In addition to being a potent vasoconstrictor on the renal vasculature [14], 20-HETE inhibits sodium resorption in proximal tubules [15] and blocks NaCl transport in the thick ascending limb [16]. Since PPARα and cytochrome P-450 are mainly co-located in the renal proximal tubules and the thick ascending limb [13], the predominant effect of PPARα agonist on tubular function may in part explain the
anti-hypertensive effect observed in some animal models such as Dahl salt-sensitive rats [17]. In humans, the PPARα agonist impact on blood pressure and natriuresis is less clear.

**Evidence of renal protection by PPARα agonists in humans**

Clinical trials using fibrates in humans for providing renal protection are primarily represented by the Diabetes Atherosclerosis Intervention Study (DAIS) and, more recently, the Fenofibrate intervention and events lowering in diabetes (FIELD) study.

The DAIS extension study compared fenofibrate with placebo in type 2 diabetes (T2D) patients [18]. For the 314 diabetic patients without nephropathy who continued to take fenofibrate or placebo over a 5-year period, the respective rates of worsening albumin excretion were 8 and 18% (P < 0.05). This benefit was predominantly attributed to blocking the progression from normal albuminuria to microalbuminuria.

The FIELD study was a randomized controlled trial that included 9795 T2D patients treated for 5 years with fenofibrate or a placebo [19]. Its results indicate that the progression from normal albuminuria to microalbuminuria or from microalbuminuria to macroalbuminuria was significantly lower for the fenofibrate group than those taking the placebo 10 and 11%, respectively [19].

**Renal effects of PPARγ agonists**

PPARγ are strongly expressed in adipose tissue and their activation stimulates preadipocyte differentiation. Moreover, PPARγ agonists improve insulin sensitivity and glucose homoeostasis by promoting uptake and storage of free fatty acids in adipose tissue, and by modifying adipocyte-derived signalling molecules, also called adipocytokines: by decreasing release of prodiabetic adipocytokines, including tumour necrosis factor-α (TNFα), interleukin (IL)-6, leptin and resistin and increasing the circulating concentration of the anti-diabetic adipocytokine adiponectin. However, the anti-diabetic effect of PPARγ agonists is mostly associated with a weight gain of ~2 kg and a trend toward the onset of oedema, with fluid retention and lower blood pressure [20]. In renal tissue, PPARγ are predominantly expressed in collecting ducts and, to a lesser extent, in glomeruli, mesangial cells, proximal tubules and the renal microvasculature [2].

**PPARγ agonist-induced fluid retention and lower blood pressure**

In ~7% of treated patients, PPARγ agonists are associated with fluid retention, haemodilution, decreased sodium fractional excretion and diuresis, lower blood pressure, higher vascular permeability and onset of oedema [20].

**Mechanism of PPARγ agonist-induced fluid retention**

Fluid retention results mainly from primary renal sodium retention induced by PPARγ agonists. Indeed, in vitro, PPARγ agonists enhance the number of luminal amiloride-sensitive epithelial Na⁺ channels (ENaC) located on the apical membrane of a cultured cell line derived from human cortical collecting ducts [21]. These enhanced ENaC activities are abolished by a selective PPARγ antagonist.

The pathophysiological relevance of PPARγ agonists in an in vivo experimental model of sodium renal retention in mice was demonstrated later by Guan et al. [22]. Early weight gain was blocked by the collecting duct-specific diuretic amiloride and was also prevented by specific PPAR-gene deletion from the collecting ducts in those mice. In the same study, the authors evaluated amiloride-sensitive Na⁺-channel activity in cultured inner medullary collecting duct (IMCD) cells from mice by measuring their radiolabelled sodium [23Na]-flux absorption. They demonstrated that PPARγ agonists significantly increased Na⁺-flux absorption and that this increase was completely blocked by a specific PPARγ antagonist. However, in IMCD cells derived from PPAR gene-deleted mice, Na⁺-flux absorption was not stimulated by pioglitazone.

It is important to retain the fact that oedema results not only from fluid retention, but also, in part, from increased capillary permeability due to lowered insulin resistance. Pertinently, insulin favours capillary permeability and sodium renal retention leading to oedema [23]. PPARγ agonists also directly increase capillary permeability by enhancing vascular VEGF production [24].

**PPARγ agonist-induced mechanisms lowering blood pressure**

Experimental and clinical studies have shown that PPARγ agonists can significantly lower blood pressure. This effect is particularly dramatic in animal models of insulin resistance. Mechanisms by which blood pressure falls remain to be clarified. However, in several insulin-resistant animals, the blood pressure decline may be partly due to increased insulin sensitivity. Alternatively, peripheral vasodilation might be explained by the involvement of three mediators: nitric oxide release from endothelial cells, excess syntheses of VEGF and vasodilating prostaglandin [24,25]. PPARγ agonists also directly induce vasodilatation secondary to inhibition of extracellular Ca²⁺ uptake via calcium channels [26]. Finally, numerous observations support the direct blockade of the angiotensin II type-1 receptors by PPARγ agonists [27].
Anti-inflammatory, anti-proliferative and anti-fibrotic properties of PPARγ agonists

In vitro effects on mesangial cells

Activation of PPARγ expressed on cultured mesangial cells by pharmacological ligands, like troglitazone, or the natural ligand, 15-deoxy-delta-prostaglandin J2 (15d-PGJ2), decreases mesangial cell proliferation and expression of smooth muscle α-actin, a marker of myofibroblast activation [28]. Furthermore, PPARγ agonists inhibited the enhanced PAI-1 expression (considered a potent profibrotic factor contributing to glomerulosclerosis) by cultured mesangial cells incubated with angiotensin II [29]. Stimulation of mesangial cell proliferation by platelet-derived growth factor (PDGF) was also blocked by PPARγ agonists [30].

In addition to its anti-proliferative properties, PPARγ ligands exerted direct anti-fibrotic actions on mesangial cells by inhibiting type I collagen expression [31] and by suppressing expression of TGFβ1-mediated smooth muscle α-actin, fibronectin and PAI-1 through an increase of hepatocyte growth factor (HGF) synthesis [32].

In vitro effects on renal tubule cells

Exposure of opossum kidney cells, used as an in vitro model of proximal tubule cells, to low-density lipoprotein (LDL) or to albumin led to an increased production of monocyte chemotactic protein-1 (MCP-1) and TGFβ1 which was reversed in the presence of pioglitazone [33]. Furthermore, prior addition of pioglitazone to the culture further enhanced albumin uptake by tubule cells but it was no longer associated with an exaggerated inflammatory or profibrotic cytokine response. Hence, it could be hypothesized that in vivo proteinuria would be reduced by enhanced proximal tubular uptake of albumin without potential deleterious effects on the tubulointerstitium.

Exposure of the human proximal tubule cell line, HK-2 to high glucose is associated with PPARγ up-regulation. The latter is probably a protective response, as it was also associated with MCP-1 gene down-regulation and consequently, less of the inflammatory protein. This effect was reproduced by a PPARγ agonist, which further decreased AP-1 and TGFβ1. This anti-inflammatory response was associated with anti-proliferative and proapoptotic effects [34].

Diabetic animal models with nephropathy

Most of the results from studies where different diabetic models with nephropathy and proteinuria were treated with PPARγ agonists strongly suggest a renal protective effect of the PPARγ agonists, through a mechanism independent of their insulin-sensitizing action. In the Zucker fatty (fa/fa) rat, a prediabetic insulin-resistant syndrome model with nephropathy and proteinuria, the PPARγ agonist delays the onset of nephropathy and proteinuria and slows down the development of renal dysfunction because of the correction of metabolic abnormalities, but probably also through a direct renal effect. Indeed, proteinuria appearance in untreated Zucker fatty control rats coincided with the development of hypertension. This implies that the renal protection of the agonist PPARγ must be explained by another mechanism than lowering blood pressure [35].

Similarly, in the inbred obese ZDF rat model of severe T2D with extensive kidney damage, angiotensin-converting enzyme inhibition (ACEI) or PPARγ agonists alone significantly prevented proteinuria and impaired renal clearance, and protected against structural damage of glomerular and tubulointerstitial tissues [36]. However, treatment with PPARγ agonists was more protective than ACEI, as assessed at 6 months of age by lower proteinuria, less glomerulosclerosis and less macrophage/monocyte tubulointerstitial infiltration. Having given the wide range of PPARγ-agonist actions, numerous mechanisms to explain their superior renal protection can be envisaged. These mechanisms are extensions of PPARγ-agonist metabolic actions on glycaemia control and the lowering hyperlipidaemia, but probably also reflect a direct anti-inflammatory, anti-proliferative and anti-fibrotic action of these drugs on the kidney [36].

Finally and moreover, in the Sprague–Dawley rat model of streptozotocin-induced diabetes, troglitazone administration started a few days after streptozotocin was able to prevent glomerular hyperfiltration, albuminuria and the enhanced build-up of extracellular matrix proteins and TGFβ1 in glomeruli, despite the absence of effect on blood pressure or glucose levels [37].

Non-diabetic animal models with nephropathy

In the different non-diabetic animal models with nephropathy such as renal ischaemia–reperfusion-induced injury in Wistar rats [38], ciclosporin A (CsA)-induced renal injury in Sprague–Dawley rats [39], renal dysfunction induced by sepsis in mice [40] and the 5/6-nephrectomy in Sprague–Dawley rats [41], prior administration of PPARγ agonists constantly generated a protective effect, as demonstrated by less interstitial inflammatory cell infiltration, interstitial fibrosis and proinflammatory mediators compared with untreated controls [38–41]. All those beneficial effects were independent of the PPARγ agonist’s action on lipid and glucose homeostases.

Evidence of renal protection by PPARγ agonists in humans

PPARγ agonists decrease UAE

Several studies have demonstrated that PPARγ agonists can prevent proteinuria in humans.

Lebovitz et al. [42] conducted a randomized trial on 493 T2D patients assigned to receive rosiglitazone or placebo for 26 weeks. They reported a statistically significant reduction of the urinary albumin/creatinine ratio (UACR) from baseline for rosiglitazone-treated patients, and that UACR was ~30% lower in that group compared with the placebo group [42].

Except in a study where the sulfonylurea gliclazide and the thiazolidinedione pioglitazone significantly and similarly lowered the UACR of T2D patients treated for 12 weeks with either molecule [43], most of the studies provide superior benefits with PPARγ agonists compared with other anti-diabetic drugs on UACR. In this way, Bakris et al. [44] found a significant UACR reduction from baseline for T2D patients treated for 52 weeks with rosiglitazone, unlike the sulfonylurea glibenclamide-treated group, which achieved no beneficial effect in terms of microalbuminuria reduction. The discrepancy between the microalbuminuria outcomes could not be explained by glucose-homeostasis control, which was similar for both the groups, but might be attributable, at least in part, to the strong relationship between UACR and blood-pressure reduction obtained only in the rosiglitazone-treated group. Hence, in addition to blood glucose control, rosiglitazone exerts a beneficial effect on microalbuminuria probably mediated, at least in part, through blood-pressure reduction. Furthermore, it cannot be excluded that the beneficial effect observed could be a consequence of other mechanisms acting directly on renal vascular protection, for example, reversal of insulin resistance (which is associated with a cluster of metabolic abnormalities including elevated PAI-1, prothrombotic and proinflammatory states...) [45]. Those results were supported by Nakaruma et al. [46], who treated 32 T2D patients for 12 months with the glibenclamide or the thiazolidinedione troglitazone and observed significant reductions of microalbuminuria only in troglitazone-treated patients. Similarly, two studies [47,48] compared the efficacies of troglitazone or pioglitazone, respectively, vs metformin on UACR in T2D patients and also found significant UACR reductions with troglitazone (~40% after 4 weeks of treatment) or with pioglitazone (19% after 52 weeks of treatment) but no change with metformin, despite comparable correction of blood-glucose levels. The lower UAE obtained with PPARγ agonists, like pioglitazone, could also be explained, in part, by increased renal proximal tubule uptake of filtered albumin [33].

In contrast, the results of the prospective PROACTIVE trial in evaluating pioglitazone efficacy against macrovascular events were very disappointing. That prospective study included 5238 T2D patients at high risk of cardiovascular complications who were treated with pioglitazone or with classical glucose-lowering drugs for 34.5 months; no information was provided concerning the impact on micro- and macroalbuminuria [49].

Nephroprotective effect of the PPARγ2 polymorphism Pro12Ala among T2D patients

The risk of developing diabetic nephropathy is likely to be, at least partly, genetically determined, because not all patients have the same renal outcome, even when they have comparable degrees of metabolic dysregulation. Microalbuminuria is a harbinger of deleterious cardiovascular and renal outcomes. Since clusters of diabetic nephropathy and higher UAE rates are found among T2D patients’ children, it is logical to look for a link between developing diabetic nephropathy and genetic background, for example, to search genetic polymorphism in the PPARγ gene that could be associated with diabetic nephropathy. Results from the Berlin diabetes mellitus study [50] revealed that, among T2D patients, those carrying the PPARγ2 Pro12Ala allele had significantly lower UAE and tended to develop proteinuria less frequently. Those observations seem to indicate a protective effect of the ProAla12 allele in relation to diabetic nephropathy.

Renal effects of PPARβ/δ agonists

Unlike PPARα and PPARγ agonists, respectively, fibrates and thiazolidinediones, physiological functions of PPARβ/δ agonists remain elusive and are currently being investigated. Beyond their pleiotropic activities on reproductive processes, cell immunity, skin wound-healing and tumorigenesis [51], PPARβ/δ are highly involved in the pathophysiology of the metabolic syndrome, more precisely, in preadipocyte differentiation and regulation of fatty acid β oxidation in skeletal muscles.

In the kidney, PPARβ/δ agonists are suspected of being intimately involved in energy-metabolism regulation, because PPARβ/δ mRNA in the kidney is dramatically down-regulated after an overnight fast and rapidly restored to control levels upon refeeding [52]. However, in addition to their metabolic actions on the kidneys, PPARβ/δ agonists also exert nephroprotective effects. In contrast to the other isotypes, PPARβ/δ are abundantly and ubiquitously expressed in all nephron segments, more specifically in proximal straight tubules and renal medullary interstitial cells [2].

PPARβ/δ agonists provide strong renal protection in the model of ischaemic acute renal failure

PPARβ/δ agonists play a pivotal role in wound healing, cell repair and survival in the context of tissue injury. This property is well-illustrated by Letavernier et al. [53] in a mouse model of ischaemia–reperfusion-induced renal injury. Sustained ischaemia leads to epithelial cell shedding into the tubule lumen, followed by necrotic and apoptotic cell death, especially in the proximal straight tubules in the outer medulla of the kidney, particularly susceptible to ischaemia. Epithelial cells that do not die participate
in the regeneration of tubule epithelium and restoration of renal function. In PPAR β/δ-deficient mice subjected to ischaemia–reperfusion, wound-healing is delayed and renal dysfunction is exacerbated, compared with wild-type mice controls [53]. Furthermore, wild-type mice pre-treated with a specific PPAR γ agonist developed less severe ischaemia-induced tubule injury than untreated control mice. Finally, prior exposure to a PPAR β/δ agonist exerts an anti-apoptotic effect on cultured human proximal tubule epithelial cells subjected to oxidative stress [53].

**Conclusion and therapeutic perspectives**

PPARα, γ and β/δ agonists provide nephroprotective effects in numerous diabetic and non-diabetic models with nephropathy, via mechanisms including prevention of renal lipid accumulation by activation fatty acids β oxidation, anti-inflammatory, anti-fibrotic and anti-apoptotic properties. However, their nephroprotective effects in humans are lesser and only manifested by prevention of microalbuminuria. Particularly, PROACTIVE study results were very disappointing as they failed to demonstrate clear PPAR γ agonist protection of the kidneys.

Despite the currently unresolved discrepancies between the results of preclinical and clinical studies concerning the efficacies of these drugs on renal disease, PPAR agonists still represent a potentially attractive therapeutic way to prevent renal tissue damage in diabetic and non-diabetic nephropathies. Further studies are needed to determine and clarify the real potential renal benefits of these drugs. It would be particularly interesting to select human renal diseases with lipid accumulation, such as diabetes, obesity, cisplatin-induced acute renal failure or age-related renal disease and to analyse the potential nephroprotective impact of PPARα agonists on them. It would be also fascinating to elucidate the impact of dual-PPARα/γ or panPPARα, -γ and -β/δ agonists on renal diseases.

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**References**

24. Mudaliar S, Chang AR, Henry RR. Thiazolidinediones, peripheral edema, and type 2 diabetes: incidence,

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