Peroxisome Proliferator-Activated Receptor-α Contributes to the Resolution of Inflammation after Renal Ischemia/Reperfusion Injury

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Received September 16, 2008; accepted November 6, 2008

ABSTRACT

This study was designed to elucidate the role of peroxisome proliferator-activated receptor (PPAR)-α in the development of inflammation after ischemia/reperfusion injury of the kidney. We have evaluated the effects of ischemia/reperfusion on renal dysfunction, injury, and inflammation in wild-type mice or mice in which the gene for PPAR-α has been deleted [PPAR-α(−/−)] and then treated with the PPAR-α agonist fenofibrate. Mice were subjected to bilateral renal ischemia (30 min) and reperfusion (24 h) and received fenofibrate (3 mg/kg i.p.) before reperfusion. Plasma creatinine, urea, and aspartate aminotransferase were all used as indicators of renal dysfunction and injury. Kidneys were used for histological and immunohistochemical analysis and markers of inflammation. Fenofibrate significantly attenuated the degree of renal dysfunction, injury, and inflammation caused by ischemia/reperfusion injury. The degree of renal dysfunction, injury, and inflammation caused by ischemia/reperfusion was also significantly augmented in PPAR-α(−/−) mice compared with their wild-type littermates. It is interesting that fenofibrate did not protect PPAR-α(−/−) mice against ischemia/reperfusion injury. Therefore, we propose that ligands of PPAR-α may be useful in the treatment of renal ischemia/reperfusion injury and that endogenous PPAR-α limits the degree of renal dysfunction, injury, and inflammation associated with ischemia/reperfusion injury.

Peroxisome proliferator-activated receptor (PPAR)-α is a member of the nuclear receptor superfamily of ligand-dependent transcription factors related to retinoid, steroid, and thyroid hormone receptors (Evans, 1988). The structure of PPAR-α (and PPAR-γ and PPAR-β) consists of an amino-terminal region that allows for ligand-independent activation and constitutive activity on the receptor and is negatively regulated by phosphorylation. This region is followed by a DNA binding domain (two zinc finger motifs separated by a linker region) and the carboxyl-terminal ligand binding domain (Moras and Gronemeyer, 1998). The role of PPAR-α expression in the kidney is not yet fully understood, but it is known that PPAR-α expression predominates in the proximal convoluted tubule and the thick limb of the loop of Henle, which could contribute to dietary lipid-induced gene expression of peroxisomal and mitochondrial fatty acid oxidation enzymes in these segments (Guan et al., 1997; Ouali et al., 1998).

We have shown previously that PPAR-α ligands protect the rat kidney against ischemia/reperfusion injury (IRI) (Sivaramakrishnan et al., 2002), which may be explained by the ability of PPAR-α to sustain the balance between energy production and utilization in the kidney by regulating the genes involved in fatty acid oxidation (Portilla et al., 2000). In fact, activation of PPAR-γ or PPAR-β also confers protection after renal IRI (Chatterjee et al., 2004; Letavernier et al., 2005). Given that no single high-affinity natural ligand has been identified for PPAR-α, it thus has been proposed that a physiological role of the receptor may be to sense the total flux of dietary

ABBREVIATIONS: PPAR, peroxisome proliferator-activated receptor; IRI, ischemia/reperfusion injury; WT, wild type; KO, knockout; FENO, fenofibrate; PBS, phosphate-buffered saline; PAR, poly(ADP-ribose); PARP, poly(ADP-ribose) polymerase; ICAM, intercellular adhesion molecule; MDA, malondialdehyde; MPO, myeloperoxidase; TNF, tumor necrosis factor; IL, interleukin.
fatty acids in key tissues. The actions of PPAR-α have been confirmed with the help of PPAR-α-deficient mice [PPAR-α(−/−)] and PPAR-α ligands such as synthetic fibrate drugs (clofibrate, bezafibrate, and fenofibrate). Synthetic PPAR-α ligands have been shown to regulate inflammatory responses (Lovett-Racke et al., 2004) and we (and many others) have clearly demonstrated that PPAR-α(−/−) mice have abnormally prolonged responses to different inflammatory stimuli (Devchand et al., 1996; Staels et al., 1998; Cuzzocrea et al., 2004b; Genovese et al., 2005; Park et al., 2006).

IRI occurs as a result of damage to the kidney after blood restoration subsequent to a loss of blood flow to the kidney. Renal IRI represents a clinically relevant problem associated with septic shock, coronary bypass surgery, and renal transplantation, which are commonly used to re-establish blood flow and minimize renal damage because of renal ischemia. However, the role of exogenous and endogenous PPAR-α ligands in acute kidney injury has yet to be fully characterized. Thus, in the present study, we have decided to examine the effects of endogenous and exogenous PPAR-α ligands on several inflammatory responses known to be involved in renal IRI.

Materials and Methods

Experimental Protocol

Forty male 129SvJ wild-type mice and 40 male PPAR-α(−/−) mice (25–30 g) were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice received a standard diet and water ad libitum, and studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Mice were anesthetized using chloral hydrate (125 mg/kg i.p.) and core body temperature maintained at 37°C using a homeothermic blanket. After performing a midline laparotomy, mice were then divided into the following eight groups.

Ischemia/Reperfusion Injury Wild-Type Group. This group included IRI WT mice, which underwent renal ischemia for 30 min followed by reperfusion for 24 h (n = 10).

Ischemia/Reperfusion Wild-Type + Fenofibrate Group. This group included IRI WT + FENO mice, which underwent renal ischemia for 30 min followed by reperfusion for 24 h and were administered fenofibrate (3 mg/kg i.p.) 5 min before reperfusion (n = 10).

Ischemia/Reperfusion Injury PPAR-α(−/−) Group. This group included IRI KO mice, which underwent renal ischemia for 30 min followed by reperfusion for 24 h (n = 10).

Ischemia/Reperfusion Injury PPAR-α(−/−) + Fenofibrate Group. This group included IRI KO + FENO mice, which underwent renal ischemia for 30 min followed by reperfusion for 24 h and were administered fenofibrate (3 mg/kg i.p.) 5 min before reperfusion (n = 10).

Sham Wild-Type Group. This group included SHAM WT mice, which were subjected to the surgical procedures described above but were not subjected to renal ischemia/reperfusion (n = 10).

Sham Wild-Type + Fenofibrate Group. This group included SHAM WT + FENO mice, which were subjected to the surgical procedures described above but were not subjected to renal ischemia/reperfusion and were administered fenofibrate (3 mg/kg i.p.) 5 min before reperfusion (n = 10).

Sham PPAR-α(−/−) Group. This group included SHAM KO mice, which were subjected to the surgical procedures described above but were not subjected to renal ischemia/reperfusion (n = 10).

Sham PPAR-α(−/−) + Fenofibrate Group. This group included SHAM KO + FENO mice, which were subjected to the surgical procedures described above but were not subjected to renal ischemia/reperfusion and were administered fenofibrate (3 mg/kg i.p.) 5 min before reperfusion (n = 10).

Mice were maintained under anesthesia for the duration of ischemia (i.e., 30 min). After performing a midline laparotomy, mice from the IRI groups were subjected to bilateral renal ischemia for 30 min, during which the renal arteries and veins were occluded using microaneurysm clamps (Chatterjee et al., 2003). The time of ischemia chosen was based on that found to maximize reproducibility of renal functional impairment while minimizing mortality in these animals (Chatterjee et al., 2003). After the renal clamps were removed, the kidneys were observed for a further 5 min to ensure reflow, after which 500 μl of saline at 37°C was injected into the abdomen, and the incision was sutured. Mice were then returned to their cages, where they were allowed to recover from anesthesia and observed for 24 h. Sham-operated mice underwent identical surgical procedures to IRI mice except that microaneurysm clamps were not applied.

Determination of Renal Injury and Dysfunction

At the end of the reperfusion period, mice were reanesthetized and 1-ml blood samples were collected via cardiac puncture, after which the heart was removed to terminate the experiment. The samples were centrifuged (9000g for 3 min) to separate plasma, from which biochemical parameters were measured within 24 h. Plasma urea and creatinine concentrations were used as indicators of impaired renal (glomerular) function (Chatterjee and Thieme, 2003). The rise in the plasma level of aspartate aminotransferase was used as an indicator of reperfusion injury (Thiemermann et al., 2003).

Histological Evaluation of Renal Injury

Kidneys were removed from rats at the end of the experimental period after tying the renal pedicle and cut in a sagittal section into two halves. These tissue samples were fixed by immersion in 10% (w/v) formaldehyde in phosphate-buffered saline (PBS; 0.01 M; pH 7.4) at room temperature for 1 week. After dehydration using graded ethanol, the tissue was embedded in Paraplast (Sherwood Medical, Mahwah, NJ), cut in fine (8 μm) sections, and mounted on glass slides. Sections were then deparaffinized with xylene, counterstained with hematoxylin and eosin, and viewed under a light microscope (Dialux 22; Leitz, Milan, Italy).

For histological scoring, renal sections were prepared as described previously and used for the assessment of renal IRI (Thiemermann et al., 2003). In brief, 100 intersections were examined for each kidney, and a score from 0 to 3 was given for each tubular profile involving an intersection: 0, normal histology; 1, tubular cell swelling, brush-border loss, nuclear condensation, with up to one third of tubular profile showing nuclear loss; 2, as for score 1 but greater than one third and less than two thirds of the tubular profile showing nuclear loss; and 3, greater than two thirds of tubular profile showing nuclear loss. The total score for each kidney was calculated by the addition of all 100 scores, with a maximum score of 300.

Immunohistochemical Localization of Nitrotyrosine

Tyrosine nitration was detected in kidney sections by immunohistochemistry as described previously (Chatterjee et al., 2003). In brief, tissues were fixed in 10% buffered formalin, and 8-μm sections were prepared from paraffin-embedded tissues. After deparaffination, endogenous peroxidase was quenched with 0.3% H2O2 in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in PBS for 20 min. Nonspecific adsorption was minimized by incubating the sections in 2% (w/v) normal goat serum in PBS for 20 min. Endogenous avidin and biotin binding sites were blocked by sequential incubation for 15 min with avidin and biotin (DBA, Milan, Italy). The sections were then incubated overnight with a 1:1000 dilution of primary anti-nitrotyrosine antibody (DBA) or with control solutions. Controls included buffer alone or nonspecific purified rabbit IgG.
Specific labeling was detected with a biotin-conjugated goat antirabbit IgG and avidin-biotin peroxidase complex (DBA).

Immunohistochemical Analysis of PAR and Intercellular Adhesion Molecule -1

Localization of PAR (indicating activation of poly(ADP-ribose) polymerase [PARP]) and intercellular adhesion molecule (ICAM)-1 in kidney sections was determined as described previously (Cockerill et al., 2001; Chatterjee et al., 2003). In brief, sections were incubated overnight at 4°C with primary anti-ICAM-1 (CD54) antibody, antinitrotyrosine, or anti-PAR antibody [1:500 (v/v) in PBS] (DBA). Controls included kidney sections incubated with buffer alone or non-specific purified IgG (DBA). After blocking endogenous avidin and biotin, specific labeling of the antigen-antibody complex was visualized using chromogen diaminobenzidine.

Malondialdehyde Measurement

Malondialdehyde (MDA) levels in kidney samples were determined as an indicator of lipid peroxidation, as described previously (Chatterjee et al., 2003). Tissues were homogenized in a 1.15% KCl solution. An aliquot of the homogenate was added to a reaction mixture containing 200 μL of 8.1% SDS, 1500 μL of 20% acetic acid, pH 3.5, 1500 μL of 0.9% thiobarbituric acid, and 700 μL of distilled water. The mixture was then boiled for 1 h at 95°C and centrifuged at 3000g for 10 min. The absorbency of the supernatant was measured by spectrophotometry at 550 nm.

Myeloperoxidase Activity

MPO activity in kidney samples was determined as an index of neutrophil accumulation, as described previously (Chatterjee et al., 2003). Kidneys were homogenized in a solution containing 0.5% hexa-decyl-trimethyl-ammonium bromide and 10 mM 3-(N-morpho)-propylsulfonic acid dissolved in 80 mM sodium phosphate buffer, pH 7, and centrifuged for 30 min at 20,000g at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (16 mM) and 1 mM hydrogen peroxide. The rate of change in absorbance was measured by a spectrophotometer at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 nmol peroxide/min at 37°C and was expressed in units per gram weight of wet tissue.

Polymorphonuclear Leukocyte Influx into Renal Tissues

Because it has become apparent that the MPO and naphthol-AS-D-chloracetate esterase assays can cross-react with monocytes and macrophages (Yseaert et al., 2000), standard hematoxylin-eosin staining was performed to estimate the presence of polymorphonuclear leukocytes, based on the morphology of the nucleus. The total number of infiltrating leukocytes (e.g., neutrophils and mononuclear cells) in cortical interstitial spaces was assessed quantitatively by counting the number of polymorphonuclear leukocytes in 20 high-powered fields.

Measurement of Cytokines

Frozen kidneys were homogenized as described previously (Patel et al., 2005a) in PBS containing 2 mM phenylmethylsulfonyl fluoride, and tissue levels of TNF-α and IL-1β were evaluated in duplicate using colorimetric commercial enzyme-linked immunosorbent assay kits (Calbiochem, La Jolla, CA) according to the manufacturer’s instructions.

Materials

Unless otherwise stated, all compounds used in this study were purchased from Sigma Chemical (Poole, Dorset, UK). Fenofibrate (Procter & Gamble UK Ltd., Nottingham, UK) was prepared in 10% (v/v) dimethyl sulfoxide. All solutions used were prepared using nonpyrogenic saline [0.9% (w/v) NaCl; Baxter, Newbury Park, UK].

Statistical Analysis

All values described in the text and figures are expressed as mean ± S.E.M. for N observations. One-way analysis of variance with Bonferroni’s post hoc test was performed on all biochemical data using GraphPad Prism version 5.01 for Windows (GraphPad Software Inc., San Diego, CA; http://www.graphpad.com), and a P value of less than 0.05 was considered to be significant.

Results

Renal Dysfunction in Mice Treated with Fenofibrate and in PPAR-α(−/−) Mice: Plasma Creatinine and Urea. Compared with sham-operated mice, IRI caused a significant increase in the plasma levels of creatinine and urea in wild-type (control) mice (Fig. 1, A and B). Administration of fenofibrate to wild-type mice subjected to IRI significantly attenuated the renal dysfunction caused by IRI in wild-type mice alone (Fig. 1, A and B). The increases in the plasma levels of creatinine and urea seen in PPAR-α(−/−) mice subjected to IRI were significantly higher than those seen in their wild-type littermates (Fig. 1, A and B). However, the degree of protection from renal dysfunction observed with fenofibrate in wild-type mice was not seen with fenofibrate in PPAR-α(−/−) mice (Fig. 1, A and B).

Renal Injury and Tubular Dysfunction in Mice Treated with Fenofibrate and in PPAR-α(−/−) Mice: Histological Assessment. Compared with sham-operated mice (Figs. 1C and 2, A and F), histological examination of kidneys and plasma aspartate aminotransferase obtained from wild-type (control) mice subjected to IRI demonstrated a significant degree of renal injury (Figs. 1C and 2, B and F). Specifically, kidneys obtained from these animals exhibited degeneration of tubular structure, tubular dilatation, swelling and necrosis, luminal congestion, and eosinophilia. In contrast, renal sections obtained from wild-type mice treated with fenofibrate that underwent IRI demonstrated a marked reduction in the severity of these histological features of renal injury (Figs. 1C and 2, C and F), compared with kidneys obtained from wild-type mice subjected to IRI. Renal sections obtained from PPAR-α(−/−) mice that underwent IRI demonstrated a marked increase in the severity of the above histological features of renal injury (Figs. 1C and 2, D and F), compared with kidneys obtained from wild-type mice subjected to IRI only. However, the degree of protection from renal injury and tubular dysfunction observed with fenofibrate in wild-type mice was not seen with fenofibrate in PPAR-α(−/−) mice (Figs. 1C and 2, E and F).

Renal Inflammation in Mice Treated with Fenofibrate and in PPAR-α(−/−) Mice: MDA Levels, MPO Activity, and Polymorphonuclear Neutrophil Counts. Compared with sham-operated mice, the kidneys obtained from wild-type mice subjected to IRI demonstrated a significant increase in MDA levels (Fig. 3). Administration of fenofibrate to wild-type mice subjected to IRI significantly attenuated the rise in MDA levels caused by IRI in wild-type mice alone (Fig. 3). The increases in the tissue level of MDA seen in PPAR-α(−/−) mice subjected to IRI were significantly higher than those seen in their wild-type littermates (Fig. 3). However, the degree of protection from lipid peroxidation observed with fenofibrate in wild-type mice was not seen with fenofibrate in PPAR-α(−/−) mice (Fig. 3).

Compared with sham-operated mice, the kidneys obtained...
from wild-type mice subjected to IRI demonstrated a significant increase in MPO activity (Fig. 4A) and, thus, neutrophil infiltration (Fig. 4B). Administration of fenofibrate to wild-type mice subjected to IRI significantly attenuated the rise in MPO activity and neutrophil infiltration caused by IRI in wild-type mice alone (Fig. 4). The increases in neutrophil infiltration seen in PPAR-α(/−) mice subjected to IRI were significantly higher than those seen in their wild-type littermates (Fig. 4). However, the degree of protection from neutrophil infiltration observed with fenofibrate in wild-type mice was not seen with fenofibrate in PPAR-α(/−) mice (Fig. 4).

**Renal Inflammation in Mice Treated with Fenofibrate and in PPAR-α(/−) Mice: Nitrotyrosine Formation.** Compared with sham-operated mice (Fig. 5A), immunohistochemical analysis of kidney sections obtained from wild-type mice subjected to I/R demonstrated a positive staining for nitrotyrosine (Fig. 5B). In contrast, administration of fenofibrate to wild-type mice subjected to IRI demonstrated no positive staining for nitrotyrosine (Fig. 5C). The marked positive stainings for nitrotyrosine seen in PPAR-α(/−) mice subjected to IRI were significantly higher than those seen in their wild-type littermates (Fig. 5D). However, the degree of protection from nitration of proteins observed with fenofibrate in wild-type mice was not seen with fenofibrate in PPAR-α(/−) mice (Fig. 5E).

**Renal Inflammation in Mice Treated with Fenofibrate and in PPAR-α(/−) Mice: ICAM-1 Expression and PARP Activation.** Compared with sham-operated mice (Figs. 6A and 7A), kidneys obtained from wild-type mice subjected to IRI demonstrated marked staining for ICAM-1 (Fig. 6B) and PARP (Fig. 7B). In contrast, administration of fenofibrate to wild-type mice subjected to IRI demonstrated no positive staining for ICAM-1 (Fig. 6C) and PARP (Fig. 7C). The marked positive stainings for ICAM-1 and PARP formation observed with fenofibrate in wild-type mice was not seen with fenofibrate in PPAR-α(/−) mice (Figs. 6E and 7E).

**Renal Inflammation in Mice Treated with Fenofibrate and in PPAR-α(/−) Mice: Kidney TNF-α and IL-1β.** To determine whether PPAR-α may modulate the inflammatory process through the regulation of the secretion of other cytokines, we analyzed the tissue levels of TNF-α and IL-1β in PPAR-α(/−) mice and their wild-type littermates. Compared with sham-operated mice, IRI caused a significant increase in the tissue levels of TNF-α and IL-1β in wild-type mice (Fig. 8). Administration of fenofibrate to wild-type mice subjected to IRI significantly attenuated the rise of tissue levels of TNF-α and IL-1β caused by IRI in wild-type mice alone (Fig. 8). The increases in the tissue levels of TNF-α and IL-1β in PPAR-α(/−) mice subjected to IRI were significantly higher than those seen in their wild-type littermates (Fig. 8). However, the degree of cytokine production observed with fenofibrate in wild-type mice was not seen with fenofibrate in PPAR-α(/−) mice (Fig. 8).

**Discussion**

Portilla et al. (2000) previously have demonstrated significantly greater renal dysfunction after renal IRI in PPAR-α(/−) mice, as assessed by higher serum creatinine levels.
(Portilla et al., 2000). In the present study, we have extended our previous observations made in the IRI model of acute kidney injury (Sivarajah et al., 2002, 2003; Chatterjee et al., 2004; Collino et al., 2005). We have confirmed that genetic deletion of PPAR-α enhances the renal dysfunction caused by 30 min of renal ischemia and 24 h of reperfusion and that the activation of PPAR-α by the specific ligand fenofibrate attenuates the renal dysfunction, inflammation, and injury caused by renal IRI. One could argue that the effects of fenofibrate in this model are nonspecific; however, we demonstrate here that the administration of fenofibrate to PPAR-α(-/-) mice does not attenuate the renal dysfunction and injury caused by IRI compared with PPAR-α(-/-) mice subjected to renal IRI only. However, the effect of renal IRI on inflammation and injury in PPAR-α(-/-) mice and in PAPR-α(-/-) mice treated with fenofibrate has not yet been reported.

We have demonstrated a significant enhancement in the production/expression of several proinflammatory markers, such as MDA (increased lipid peroxidation), MPO (increased neutrophil infiltration), nitrotyrosine (increased nitrosative stress), ICAM-1 (increased neutrophil adherence), PARP (increased oxidative stress), TNF-α, and IL-1β after renal IRI in PPAR-α(-/-) mice; this enhancement was not attenuated by fenofibrate. All these data confirmed a well known pattern of renal injury, dysfunction, and inflammation caused by IRI of the kidney, which has been documented previously by us and many others (Paller, 1994a; Chatterjee et al., 2000b, 2003, 2004; Sheridan and Bonventre, 2001; Patel et al., 2005a,b). Moreover, our findings are in agreement with the notion that renal IRI causes tubular injury (Paller, 1994b) and secondary inflammation (Dragun et al., 2000). We report here for the first time that all of the above signs of renal injury and inflammation were significantly increased in PPAR-α(-/-) mice. This finding supports the view that endogenous and exogenous PPAR-α ligands exert potent anti-inflammatory effects and that specific PPAR-α ligands such as fenofibrate may be useful in the prevention of renal IRI.

Over the past decade, numerous other studies have highlighted the benefits of activating other PPAR isoforms in IRI. We have demonstrated that the PPAR-γ ligands rosiglitazone and ciglitazone can ameliorate IRI of the rat kidney via reduction of the expression of ICAM-1 and subsequent de-
crease in the infiltration of neutrophils into reperfused renal tissues and reduction of oxidative stress (Sivarajah et al., 2002). In the same study, we demonstrated that PPAR-γ is expressed in the rat kidney and that this expression is maintained throughout renal IRI (Sivarajah et al., 2002). In contrast, renal IRI can also result in a significant attenuation of the expression of PPAR-α in the rat kidney (Sivarajah et al., 2003). This was later confirmed when PPAR-α expression in the mouse heart was also attenuated after IRI, which is now known to be because of the generation of reactive oxygen species (Dewald et al., 2005). It is interesting that Dewald et al. (2005) found that “reactivation” of this down-regulated PPAR-α significantly increased heart dysfunction after IRI. This possibly suggests that the administration of PPAR-α agonists may only be of benefit when given either before an ischemic insult or at the onset of reperfusion as seen in this study. Therefore, it may be reasonable to assume that therapeutic administration of PPAR-α agonists is detrimental to the recovery of organs after IRI. This assumption has been recently highlighted in mice with chronic pressure overload where fenofibrate was shown to exert deleterious, pleiotropic myocardial actions in the absence of PPAR-α (Duhaney et al., 2007). The role of PPAR-β in renal IRI has only been addressed recently. As with PPAR-γ and PPAR-α, the activation of PPAR-β before reperfusion results in mice being functionally and histologically protected from renal IRI because of the activation of the antiapoptotic Akt signaling pathway (Letavernier et al., 2005). It is interesting that both PPAR-γ and PPAR-β (unlike PPAR-α) are expressed in the straight part of the proximal tubule, the area of the kidney predominantly affected by ischemic lesions; therefore, the mechanism by which PPAR-α agonists exert beneficial effects in experimental models requires further investigation.

It has been demonstrated recently that PPAR-α ligands are able to suppress the expression of C-reactive protein induced by IL-1 and fibrinogen induced by IL-6, which are the major acute-phase response proteins in humans whose plasma concentrations are elevated not only in acute but also chronic inflammatory states. The anti-inflammatory action of PPAR-α is not only restricted to these genes but applies more generally to other acute phase response genes, such as serum amyloid A, fibrinogen-α, and fibrinogen-γ (Gervois et al., 2001). In addition, activation of PPAR-α increases IκBα expression (Delerive et al., 2002), thus preventing the nuclear translocation of NF-κB, which in turn would prevent the transcription of many proinflammatory genes, including TNF-α, IL-1β, and ICAM-1, to name but a few. There is evidence that the proinflammatory cytokines TNF-α and IL-1β help to propagate the extension of a local or systemic inflammatory process (Utsumi et al., 1991; Alonzi et al., 1998), especially during sepsis, and have been used to predict mortality in patients. We also show that the inflammatory process caused by renal IRI leads to a substantial increase in the levels of both TNF-α and IL-1β in the kidney. It is
interested that the levels of these two proinflammatory cytokines were significantly higher in kidneys obtained from animals that are PPAR-α deficient, regardless of the animals being treated with fenofibrate or not. This finding suggests that in the presence of PPAR-α, the degree of inflammation, and, hence, the formation of TNF-α and IL-1β caused by renal IRI are significantly attenuated.

The mechanisms underlying the activation of neutrophils in IRI are currently insufficiently characterized. Reperfusion of an organ results in the release of oxygen free radicals; autocoids; autocrine, paracrine, and endocrine mediators; and cytokines, which result in changes to: 1) the adhesive properties of the endothelium, 2) the chemotactic attraction of cells from the blood, 3) vessel permeability, and even 4) the expression of receptors. A number of recent studies have demonstrated that the recruitment of cells into an area of inflammation may be mediated not only by C5a, leukotrienes, platelet-activating factor, or bacterially derived peptides but also by a novel group of small proteins with relatively specific chemotactic activity for neutrophil subpopulations (Caramelo and Alvarez Arroyo, 1998). We report in the present study that endogenous and exogenous PPAR-α ligands are able to significantly attenuate the increase in neutrophil infiltration as assessed by the specific granulocyte enzyme MPO, neutrophil counts, and kidney tissue damage. Neutrophils that are recruited into the tissue can contribute to tissue destruction by the production of reactive oxygen metabolites, granzymes, and cytokines that further amplify the inflammatory response by their effects on macrophages and lymphocytes. Neutrophils have been shown to express PPAR-γ, suggesting a potential role of this transcription factor in neutrophil function (Greene et al., 1995). We have shown previously that PPAR-α ligands may modulate neutrophil-endothelial cell interactions during inflammation through regulation of endothelial adhesion molecules (Cuzzocrea et al., 2004a). Thus, we propose that the reduction of neutrophil infiltration caused by endogenous and exogenous PPAR-α ligands contributes to the reduction of the organ injury caused by renal IRI.

IRI of the kidney leads to an enhanced formation of reactive oxygen species and peroxynitrite (Chatterjee et al., 2000a, 2003). The biological activity and decomposition of peroxynitrite are very much dependent on the cellular or chemical environment, and these factors influence its toxic potential (Beckman et al., 1990; Rubbo et al., 1994). We demonstrate here that renal IRI of wild-type mice leads to a subsequent increase in the degree of nitrosylation of proteins in the kidney. In contrast, the degree of staining for nitrotyrosine was markedly increased in PPAR-α(-/-) mice and markedly attenuated in wild-type mice administered fenofibrate. There is recent evidence that certain reactions can also
induce tyrosine nitration, e.g., the reaction of nitrate with hypochlorous acid and the reaction of MPO with hydrogen peroxide can lead to the formation of nitrotyrosine (Eiserich et al., 1998). Increased nitrotyrosine staining is considered, therefore, as an indication of increased nitrosative stress. Thus, our results suggest that the degree of nitrosative stress caused by renal IRI is increased in kidneys from animals that are unable to produce endogenous ligands for PPAR-α. The enhanced generation of reactive oxygen species during renal IRI may not only promote the generation of peroxynitrite but also cause tissue injury secondary to protein denaturation, DNA damage, and peroxidation of membrane lipids (Szabo et al., 1997). We demonstrate here that the degree of lipid peroxidation (determined as MDA formation within the kidney) is enhanced in PPAR-α(-/-) mice. This finding supports the view that endogenous ligands for PPAR-α attenuate the degree of oxidative stress.

Various studies have demonstrated clearly that reactive oxygen species produce strand breaks in DNA, which triggers energy-consuming DNA repair mechanisms and activates the nuclear enzyme PARP, resulting in the depletion of its substrate NAD and, hence, a reduction in the rate of glycolysis. Because NAD functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP. This process has been termed “the PARP suicide hypothesis” (Szabó et al., 1997). Further evidence indicates that the activation of PARP also plays an important role in inflammation (Chatterjee et al., 2000b; Patel et al., 2005b). We demonstrate here that endogenous and exogenous PPAR-α ligands attenuated the activation of PARP during renal IRI. Thus, we propose that the anti-inflammatory effects of endogenous and exogenous PPAR-α ligands may be, at least in part, because of the prevention of the activation of PARP.

In conclusion, this study demonstrates that endogenous ligands for PPAR-α attenuate the degree of renal injury, dysfunction, and inflammation caused by IRI. This finding indicates that: 1) PPAR-α can act as an anti-inflammatory transcription factor in renal disorders associated with IRI and 2) that strategies aimed at enhancing the activity and/or the effects of PPAR-α may be useful in conditions associated with renal ischemia and inflammation. Our finding that the renal injury, dysfunction, and inflammation caused by IRI is augmented in PPAR-α(-/-) mice indicates that the genera-
tion of endogenous PPAR-α ligands limits IRI of the kidney. The nature of these endogenous ligands for PPAR-α has been discussed (Moraes et al., 2006) but is not entirely clear.

References


