Anti-inflammatory effect of fibrate protects from cisplatin-induced ARF

Shenyang Li,1 Neriman Gokden,1 Mark D. Okusa,2 Renu Bhatt, and Didier Portilla1

Division of Nephrology, Departments of Internal Medicine and Pathology, University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System, Little Rock, Arkansas; and 2Division of Nephrology Department of Medicine University of Virginia, Charlottesville Virginia

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Li, Shenyang, Neriman Gokden, Mark D. Okusa, Renu Bhatt, and Didier Portilla. Anti-inflammatory effect of fibrate protects from cisplatin-induced inflammatory responses during ARF. Mice subjected to a single intraperitoneal injection of cisplatin developed ARF at day 3. Cisplatin increased mRNA and protein expression of TNF-α, RANTES, and also upregulated endothelial adhesion molecules ICAM-1/VCAM-1 and chemokine receptors CCR1/CCR5. Cisplatin also led to neutrophil infiltration in the corticomedullary region. Pretreatment of wild-type mice with WY-14,643, a fibrate class of PPAR ligands, before cisplatin significantly suppressed cisplatin-induced upregulation of cytokine/chemokine expression, prevented neutrophil accumulation, and ameliorated renal dysfunction. In contrast, treatment with PPARα ligand before cisplatin did not prevent cytokine/chemokine production, neutrophil accumulation, and did not protect kidney function in PPARα null mice. In addition, we observed that cisplatin-induced NF-κB binding activity in nuclear extracts from wild-type mice was markedly reduced by treatment with PPARα ligand. These results demonstrate that PPARα exerts an anti-inflammatory effect in kidney tissue by a mechanism that includes inhibition of NF-κB DNA binding activity, and this effect results in inhibition of neutrophil infiltration, cytokine/chemokine release, and amelioration of cisplatin-induced ARF.

PPARα; fibrate; cisplatin-induced ARF

CISPLATIN IS ONE OF THE MOST common antitumor agents used in the chemotherapy of malignancies. Nephrotoxicity is frequent and is the major limitation in cisplatin-based chemotherapy (1). There are several mechanisms that contribute to renal dysfunction following exposure to cisplatin: direct tubular toxicity in the form of apoptosis and necrosis (1), vascular factors (29, 42), and inflammation (36–37). Recent studies suggest that similar to ischemia-reperfusion, cisplatin injury can cause endothelial cell dysfunction and neutrophil infiltration, which then leads to the release of cytokines/chemokines (4, 18, 26, 32, 36–37). Furthermore, infiltration of circulating leukocytes triggered by locally secreted cytokines/chemokines such as tumor necrosis factor-α (TNF-α) and regulated on activation Normal T cell Expressed and Secreted (RANTES) is thought to contribute to the initiation and progression of renal disease (36–37). Two recent studies support the role of chemokine receptors in acute renal failure (ARF). In the first study, it was shown that the use of the CCR1 antagonist BX471 was similar to cyclosporin in its ability to prevent acute renal allograft rejection and improved survival (25), and in the second study the investigators showed that CCR1 but not CCR5 was required for leukocyte recruitment and fibrosis after unilateral ureteral obstruction in mice (16).

Work from our laboratory has established a causal relationship between cisplatin-mediated inhibition of substrate oxidation and the development of proximal tubule cell death. We have shown that gene deletion of peroxisome proliferator-activated receptor-α (PPARα) increases kidney susceptibility to ischemia-reperfusion and cisplatin-induced ARF (33, 35). Furthermore, treatment of wild-type mice with PPARα ligands inhibited the induction of both fatty acid and glucose oxidation, resulting in significant reduction in cisplatin-induced proximal tubule cell death and prevention of cisplatin-induced organ dysfunction (28). The potential role that PPARα could play in the inflammatory reaction associated with cisplatin-induced ARF is currently unknown.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that regulate a diversity of functions such as lipid, glucose metabolism, and adipogenesis. Recent evidence suggests that PPARs play an important role in the modulation of inflammatory responses (9). Three different isotypes of PPARs family have been identified: PPARα, PPARβ, and PPARγ. PPARα and PPARγ are nuclear receptors that, besides being involved in the control of fatty acids and glucose metabolism, share potent anti-inflammatory properties (11). They act as anti-inflammatory molecules by repressing the activity of transcription factors, such as nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT), nuclear factor of activated T cells (NFAT), and activator protein-1 (AP-1) (10, 39, 43, 44).

In the present study, we examined the profile of renal cytokine gene expression in cisplatin nephrotoxicity, and, more importantly, we studied the role of PPARα in the pathogenesis of cisplatin-induced ARF. We used ribonuclease protection assays and quantitative RT-PCR to measure mRNA levels of cytokines/chemokines, chemokine receptors, cell adhesion molecules ICAM-1 and VCAM-1, and gel-shift analysis to measure NF-κB binding activity in nuclear extracts isolated from mice treated with cisplatin. To evaluate the possible role of PPARα in cisplatin nephrotoxicity, we administered WY-14,643 (WY), a fibrate class of PPARα ligand, to wild-type mice treated with cisplatin.

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and PPARα null mice exposed to cisplatin. Our studies demonstrate that PPARα exerts an anti-inflammatory effect on cisplatin-mediated ARF via inhibition of NF-kB DNA binding activity, and this effect results in inhibition of neutrophil infiltration, cytokine/chemokine release, and amelioration of ARF.

METHODS

In Vivo Model of Cisplatin-Induced ARF

Effect of PPARα ligand. SV129 mice ~8–10 wk old, weighing 25–30 g, were assigned to treatment groups. Animals received single intraperitoneal doses of saline (control) or cisplatin (20 mg/kg body wt). After treatment, the animals were killed and the kidneys were frozen in liquid nitrogen for RNA or protein isolation. Mice were housed in a temperature- and light-controlled environment and provided food and water. Pelleted mouse chow was prepared containing either 0 or 0.1% (wt/wt) WY (Cayman Chemical, Ann Arbor, MI). To investigate the effects of PPARα activation, wild-type and PPARα null mice were fed the WY-containing diet for 10 days. Blood urea nitrogen (BUN) and creatinine were measured by an enzymatic assay as previously described (28). All experimental procedures were approved by the Animal Care and Use Committee of the Central Arkansas Veterans Health Care System (Little Rock, AR) and were in accordance with the National Institutes of Health and American Physiological Society’s Guiding Principles in the Care and Use of Laboratory Animals.

Measurement of Cytokines and Chemokine Release

RNA isolation. Mice were killed following previously described experimental conditions, and the kidneys were rapidly snap-frozen in liquid nitrogen and stored at −75°C. Total RNA was extracted with TRIzol Reagent (Invitrogen Life Technologies) according to the manufacturer’s directions.

Multiprobe ribonuclease protection assay. The expression of multiple cytokine, chemokine, and chemokine receptors genes was studied by ribonuclease protection assay (RPA) using mCK-3b, mCK-5c, and mCR-5 multiprobe template sets according to the manufacturer’s instructions (BD Biosciences Pharmingen, San Diego, CA). Antisense RNA probes including housekeeping genes L32 and GAPDH were generated by in vitro transcription in the presence of [α-32P]UTP. Yeast tRNA was included in each experiment as a negative control. Twenty micrograms of total RNA were hybridized overnight with antisense RNA probes. The RNase-protected fragments were resolved in a 5% denaturing polyacrylamide gel. The dried gel was first analyzed on a 445 SI PhosphorImager with ImageQuantNT (Molecular Dynamics, Sunnyvale, CA) and then subjected to autoradiography.

Quantitative RT-PCR. Total RNA extract was treated with RQ1 RNase-free DNase (Promega) before reverse transcription (RT). RT reaction was performed at 42°C for 50 min in a total volume of 20 μl containing 5 μg RNA, 0.5 μg of oligo(dT)12-18, and 200 U of superscript II RNase H− reverse transcriptase (Invitrogen Life Technologies). Subsequently, reverse transcriptase was inactivated by incubation at 70°C for 15 min, followed by treatment with RNase H at 37°C for 30 min. PCR was performed with 1/20 of the RT reaction in a total volume of 50 μl using Taq DNA Polymerase (Invitrogen). To control for the generation of PCR products due to residual contamination of genomic DNA, an aliquot of RNA, not treated with RT, was also tested in parallel with the original samples. To compare ICAM-1 and VCAM-1 mRNA expression levels, ICAM-1 or VCAM-1 DNAs were simultaneously coamplified with 28S rRNA in the same reaction. The amplified products were measured during the exponential phase of the reaction. A series of calibration experiments was carried out in which progressive dilution of cDNA was amplified in consecutive cycle number after optimization of the reagents and conditions in the amplification reaction. Therefore, the protocol used to measure mRNA levels is in the log portion of the amplification curve. A titration curve was examined to ensure that the amplification cycle number was below the plateau phase for each gene investigated. The optimized RT-PCR cycle number depends on the initial target gene mRNA level. Amplification was performed using the following primer pairs (denaturation at 94°C for 30 s, annealing at 62°C for 25 s, and extension at 72°C for 25 s): ICAM-1 sense (5’-CAACTGGAAGCTTGTTAGCTC-3’) and antisense (5’-TAGCTTGGAATCAGAAGTCCG-3’); VCAM-1 sense (5’-TCTACCTGTCGCTTGAC-3’) and antisense (5’-ACATAAATTGCCGGAATCGTC-3’); and 28S rRNA sense (5’-TGAGCTCTCGCGTGCCCT-3’) and antisense (5’-ACATGGTCTCAACATGCAGCA-3’). The sense primer was end-labeled using [γ-32P]ATP (PerkinElmer) and T4 polynucleotide kinase. Five microfilters from the PCR reactions were resolved on a 4% acrylamide gel. The gels were dried, analyzed on a 445 SI PhosphorImager with ImageQuantNT, and then subjected to autoradiography. Results are presented as the ratio of the signal for ICAM-1 or VCAM-1 band to that of the 28S rRNA signal.

Quantitation of Protein Levels of TNF-α and RANTES by ELISA

The protein levels of TNF-α and RANTES in mouse kidney tissue were measured using commercially available cytokine-specific murine ELISA kits (Quantikine Mouse TNF-α and Quantikine Mouse RANTES kits, R&D Systems, Minneapolis, MN) according to the manufacturer’s directions. Kidney tissue was homogenized in PBS containing 0.05% Tween 20. Aliquots containing 300 μg of total protein were used for the TNF-α and the RANTES protein assay.

Histological Evaluation of Leukocyte Infiltration

For immunohistochemical studies, 5-μm sections obtained from fixed kidney tissue were subjected to antigen retrieval according to the manufacturer’s protocol (Vector Laboratories, Burlingame, CA). Then, sections were incubated with a well-characterized rat anti-mouse monoclonal antibody to murine neutrophils (7/4 clone; 1 μg/ml final concentration) followed by a biotinylated goat anti-rat secondary antibody (1:1,000 dilution). A peroxidase reaction was performed according to the manufacturer’s protocol (Vectastain ABC Elite kit). Tissue sections were covered with an aqueous-based mounting solution consisting of p-phenylenediamine (1 mg/ml) and 70% glycerol, and coverslips were applied and affixed with nail polish. Sections were viewed under a Zeiss Axioskop photofluorescence microscope. Photographs were taken with a SPOT RT Camera (software version 3.3; Diagnostic Instruments, Sterling Heights, MI). Images were imported into Adobe Photoshop (3.0) for brightness/contrast adjustment. We quantified neutrophils in the cortex and outer medulla by viewing sections under ×400 magnification. Discrete immunoreactive cells were counted and averaged from 7–10 fields/mouse kidney.

EMSA

EMSAs were performed using nuclear extracts obtained from mouse kidney tissue to examine NF-kB DNA binding activity. Nuclear fractions from mouse kidney were prepared using a modified procedure from previously described methods (6). Frozen total kidney nuclei were homogenized and then resuspended in 50 mM Tris (pH 7.4) containing protease inhibitor cocktail set 1 (Calbiochem) and 1 mM Na-orthovanadate. The suspension was centrifuged at 4,000 g for 20 min at 4°C. The resulting pellet was collected and resuspended in a lysis buffer containing 20 mM HEPES (pH 7.9), 350 mM NaCl, 20% glycerol, 1 mM MgCl2, 0.5 mM EDTA, 0.1 mM EGTA, and 1% Nonidet P-40. This lysate was then vigorously rocked at 4°C for 30 min on a shaking platform and then centrifuged at 13,000 g for 10 min at 4°C. The resulting supernatant was transferred into a small dialysis bag, and then dialyzed against 1,000-fold volume of 20 mM HEPES.
PPARα, INFLAMMATION, AND ACUTE RENAL FAILURE

PAUL R. KINSEY, ZHENGCHENG ZHAO, ROBERT J. GAFFNEY, LIANG HUANG, OLGA T. CHU, AND KAPIL C. CHANDRA

Mega, WI) DNA probes were prepared by end labeling with 32P oligonucleotide (sense strand 5’-H11032 cisplatin developed ARF at absence of cisplatin. Mice treated with a regular diet and saline (control), cisplatin, and with WY in the presence and in BUN and creatinine in PPAR

RESULTS

Protective Effects of PPARα Ligand on Cisplatin-Induced ARF

Kidney function was monitored by measuring BUN and serum creatinine for 4 days after intraperitoneal injection of saline (control and WY groups) or cisplatin (cisplatin and cisplatin+WY groups). Figure 1, A and B, present the changes in BUN and creatinine in PPARα wild-type mice treated with saline (control), cisplatin, and with WY in the presence and absence of cisplatin. Mice treated with a regular diet and cisplatin developed ARF at day 3 (BUN increased from 28 to 135 mg/dl, and creatinine increased from 0.2 to 1.2 mg/dl). The group of mice that received the WY diet and cisplatin did not develop significant ARF compared with mice treated with cisplatin alone (BUN went from 24 on day 1 to 32 mg/dl, and creatinine was unchanged at 0.2 mg/dl after 3 days of cisplatin administration). In contrast to the protective effect of the ligand on wild-type mice, PPARα null mice treated with cisplatin and WY still developed ARF at day 3 after cisplatin injection (data not shown).

Profile of Cytokine/Chemokine mRNA Expression During Cisplatin Nephrotoxicity

For measurement of cytokine/chemokine mRNA levels, we used the multiprobe RPA as described in METHODS. Mouse kidney tissue was harvested at days 1–4 after a single intraperitoneal injection of either saline (control) or cisplatin. As shown in Fig. 2A, a number of cytokine transcripts including TNF-α, IL-6, and IFN-γ mRNAs were upregulated by cisplatin. There was a 4.0-, 6.6-, and 4.0-fold increase of mRNA expression for cytokines TNFα, IL-6, and IFN-γ, respectively (P < 0.005) after 2 days of cisplatin injection compared with saline-treated mice, as shown in Fig. 2B. In most cases, the maximum levels of TNF-α, IL-6, and IFN-γ in mice treated with cisplatin were seen at day 2 but were still significantly elevated at day 4 compared with saline-treated mice. In contrast, mRNA levels of lymphotoxin α and lymphotoxin β did not show significant changes after cisplatin treatment (data not shown). Previous studies have shown that, in addition to cytokines, chemokines are also involved in the resolution and progression of renal diseases (38). Therefore, we examined the effect of cisplatin on chemokine expression in kidney tissue of mice treated with cisplatin. As shown in Fig. 3, A and B, by representative autoradiogram and PhosphorImager quantitative analysis, mRNA levels of various chemokines were increased in cisplatin-treated animals compared with saline-treated mice. Cisplatin-treated mice exhibited significant increases in mRNA levels of the following chemokines at day 2: RANTES (7.2-fold), MIP2 (12.1-fold), IP10 (6.3-fold), MCP1 (5.8-fold), and TCA3 (6.2-fold). In contrast, mRNA levels of renal MIP1 and MIP1β were not affected by cisplatin treatment (data not shown).

![Fig. 1. Effects of peroxisome proliferator-activated receptor-α (PPARα) ligand WY-14,643 (WY) on renal function in mice after single dose of cisplatin.](http://ajprenal.physiology.org/)

Fig. 1. Effects of peroxisome proliferator-activated receptor-α (PPARα) ligand WY-14,643 (WY) on renal function in mice after single dose of cisplatin. Mice were fed with either a regular diet or a diet containing 0.1% WY for 10 days before cisplatin administration. Blood urea nitrogen (BUN) and creatinine levels were measured at day 3 after either saline (control and WY) or cisplatin (cisplatin and cisplatin + WY). Values are means ± SE of at least 4 independent experiments under each condition. A and B: changes in BUN and creatinine levels, respectively, after saline or cisplatin administration. *P < 0.001 when animals were compared with control by unpaired Student’s t-test.
Our most recent studies have shown that the use of PPAR\(\gamma\)/H9251 ligands prevents the development of proximal tubule cell death during ARF by preserving peroxisomal and mitochondrial fatty acid oxidation but also by preventing the expression and translocation of proapoptotic mitochondrial endonuclease G activity (27, 28). In addition to its effects on fatty acid oxidation and antiapoptosis, recent reports suggest that PPAR\(\gamma\)/H9251 may also play a role in the regulation of cytokine gene expression in inflammation (9). Therefore, we examined the effect of PPAR\(\gamma\)/H9251 ligand on cisplatin-mediated cytokine expression in kidney tissue. As shown in Fig. 2, \(\text{C}\) and \(\text{D}\), cytokine mRNA levels of wild-type mice receiving a regular diet were significantly increased by cisplatin treatment. At day 4 there was a 2.6-, 4.9-, and 3.2-fold increase in the mRNA levels of renal TNF-\(\alpha\), IL-6, and IFN-\(\gamma\), respectively (\(P < 0.005\)) compared with saline-treated mice. In contrast, the group of wild-type mice that received the diet containing PPAR\(\gamma\)/H9251 ligand and cisplatin exhibited comparable levels of cytokine TNF-\(\alpha\), IL-6, and IFN-\(\gamma\) to the mice treated with saline alone. In addition to its modulatory role in cytokine production and inflammation, recent evidence suggests a role for PPAR\(\gamma\) in the modulation of cytokine gene expression (7, 9, 11). Therefore, in the next series of studies, we examined the effect of PPAR\(\gamma\) ligand on chemokine expression in cisplatin-treated mice. As shown in Fig. 3, \(\text{A}\) and \(\text{B}\), cisplatin induced a time-dependent increase in the mRNA levels of RANTES, MIP2, IP10, MCP1, and TCA3. In particular, RANTES mRNA levels were elevated as early as 2 days after cisplatin injection and remained elevated throughout day 4. At day 4 cisplatin induced a 7.2-fold increase in the levels of RANTES mRNA levels, an 8.6-fold increase in the level of MIP2, a 5.1-fold increase in the level of IP10, a 3.9-fold increase in the level of MCP1, and a 4.5-fold increase in the level of TCA3. As shown in Fig. 3, \(\text{C}\) and \(\text{D}\), treatment with PPAR\(\gamma\) ligand before cisplatin resulted in a significant reduction in mRNA levels of RANTES, MIP2, and MCP1 to levels comparable to saline-treated mice. Altogether, these results suggest that the protective effect of PPAR\(\gamma\) ligand against proximal tubule cell death relates not only to its metabolic and antiapoptotic effect but also to the inhibition of cytokine/chemokine production.

**Effect of PPAR\(\gamma\) Ligand on Kidney Tissue Protein Levels of TNF-\(\alpha\) and RANTES**

Our previous experiments presented in Figs. 2 and 3 demonstrated that TNF-\(\alpha\) and RANTES mRNA levels were increased in cisplatin-treated mice, results that were consistent with previous reports (36, 37). Therefore, we next examined the potential effect of PPAR\(\gamma\) ligand attenuating not only mRNA levels but also protein levels of proinflammatory RANTES and TNF-\(\alpha\). Figure 4A shows that cisplatin led to a progressive rise in RANTES protein levels from day 1 (2.6-fold) to day 4 (12.7-fold) in PPAR\(\gamma\) wild-type (+/+) mice.

**PPAR\(\alpha\) Ligand Prevents Cisplatin-Induced Increased Expression of Cytokine/Chemokines**

Our most recent studies have shown that the use of PPAR\(\alpha\) ligands prevents the development of proximal tubule cell death during ARF by preserving peroxisomal and mitochondrial fatty acid oxidation but also by preventing the expression and translocation of proapoptotic mitochondrial endonuclease G activity (27, 28). In addition to its effects on fatty acid oxidation and antiapoptosis, recent reports suggest that PPAR\(\alpha\) may also play a role in the regulation of cytokine gene expression in inflammation (9). Therefore, we examined the effect of PPAR\(\alpha\) ligand on cisplatin-mediated cytokine expression in kidney tissue. As shown in Fig. 2, \(\text{C}\) and \(\text{D}\), cytokine mRNA levels of wild-type mice receiving a regular diet were significantly increased by cisplatin treatment. At day 4 there was a 2.6-, 4.9-, and 3.2-fold increase in the mRNA levels of renal TNF-\(\alpha\), IL-6, and IFN-\(\gamma\), respectively (\(P < 0.005\)) compared with saline-treated mice. In contrast, the group of wild-type mice that received the diet containing PPAR\(\alpha\) ligand and cisplatin exhibited comparable levels of cytokine TNF-\(\alpha\), IL-6, and IFN-\(\gamma\) to the mice treated with saline alone. In addition to its modulatory role in cytokine production and inflammation, recent evidence suggests a role for PPAR\(\alpha\) in the modulation of cytokine gene expression (7, 9, 11). Therefore, in the next series of studies, we examined the effect of PPAR\(\alpha\) ligand on chemokine expression in cisplatin-treated mice. As shown in Fig. 3, \(\text{A}\) and \(\text{B}\), cisplatin induced a time-dependent increase in the mRNA levels of RANTES, MIP2, IP10, MCP1, and TCA3. In particular, RANTES mRNA levels were elevated as early as 2 days after cisplatin injection and remained elevated throughout day 4. At day 4 cisplatin induced a 7.2-fold increase in the levels of RANTES mRNA levels, an 8.6-fold increase in the level of MIP2, a 5.1-fold increase in the level of IP10, a 3.9-fold increase in the level of MCP1, and a 4.5-fold increase in the level of TCA3. As shown in Fig. 3, \(\text{C}\) and \(\text{D}\), treatment with PPAR\(\alpha\) ligand before cisplatin resulted in a significant reduction in mRNA levels of RANTES, MIP2, and MCP1 to levels comparable to saline-treated mice. Altogether, these results suggest that the protective effect of PPAR\(\alpha\) ligand against proximal tubule cell death relates not only to its metabolic and antiapoptotic effect but also to the inhibition of cytokine/chemokine production.
Similarly to RANTES and as shown in Fig. 4B, cisplatin at day 4 led to a 12.7-fold increase in TNF-α protein levels (P < 0.001) in PPARα wild-type (+/+ ) and null mice (−/−), respectively. Pretreatment with PPARα ligand WY prevented cisplatin-induced upregulation of TNF-α protein levels in PPARα wild-type mice. In contrast, pretreatment with WY did not have a significant effect on cisplatin-induced upregulation of TNFα protein levels in PPARα null mice (−/−). Figure 4C shows that cisplatin at day 4 caused a 12.7-fold increase in the protein levels of RANTES in PPARα wild-type (+/+), but also an 11-fold increase in RANTES protein levels in PPARα null mice. Pretreatment with PPARα ligand WY prevented cisplatin-induced upregulation of RANTES protein levels in wild-type mice reducing RANTES protein levels to similar values of the saline treated wild-type mice. In contrast, pretreatment with WY did not affect cisplatin-induced upregulation of RANTES protein levels in PPARα null mice (−/−), as shown in Fig. 4C. Because PPARα null mice normally do not respond to the effects of PPARα ligands, altogether our results suggest that the observed effects of PPARα ligand on preventing mRNA and protein level expression of proinflammatory TNF-α and RANTES expression were dependent on having an intact PPARα gene.

Cisplatin-Induced Chemokine Receptor Expression

Because our data show that cisplatin induces the expression of various chemokines, we next examined the effects of cisplatin on chemokine receptor expression in mouse kidney. We examined the levels of expression of various chemokine receptors, including CCR1, CCR1b, CCR2, CCR3, CCR4, and CCR5 by RPA. As shown in Fig. 5, A and B, cisplatin caused a 5.9-fold increase in the mRNA expression for CCR1 compared with saline-treated mice, as well as a moderate (2.6-fold) increase in the level of CCR5. We did not detect significant changes in mRNA expression for CCR2, CCR3, and CCR4 (data not shown).

PPARα Ligand Prevents Cisplatin-Induced Increased Expression of CCR1 mRNA Levels

The results shown above indicate that cisplatin was capable of inducing the expression of both mRNA and protein levels of RANTES in kidney tissue, and also that cisplatin upregulated the expression of RANTES receptor CCR1. Because PPARα ligand prevented cisplatin-induced upregulation of RANTES, we next examined the effect of PPARα ligand WY on chemokine receptor expression. Cisplatin induced a 5.0-fold increase in the expression of CCR1 and a 2.0-fold increase in the expression of CCR5 compared with saline-treated mice (Fig. 5, C and D). Pretreatment with WY almost completely inhibited cisplatin-induced increased expression of CCR1 and CCR5. The mRNA levels of renal CCR1 and CCR5 in mice treated with WY alone or WY plus cisplatin were almost similar to the levels in saline-treated mice.

Cisplatin-Induced Increased VCAM-1 and ICAM-1 Expression was Prevented by PPARα Ligand

Chemokines can trigger the expression of adhesion molecules on vascular endothelium and circulating platelets and
leukocytes. More specifically, previous studies have shown that P-selectins and ICAM-1 are expressed on the microvascular endothelium after ischemia-reperfusion and that the inhibition of ICAM-1 ameliorated renal injury (26). To examine the potential role of adhesion molecules in cisplatin injury, we examined the effects of cisplatin on ICAM-1 and VCAM-1 expression in kidney tissue. We found that cisplatin increased both VCAM-1 (3.8-fold) and ICAM-1 (3.4-fold) mRNA levels compared with saline-treated mice ($P < 0.05$). Pretreatment with PPAR$\alpha$ ligand inhibited the induction of both VCAM-1 and ICAM-1 expression (Fig. 6).

**Neutrophil Infiltration in Wild-Type and PPAR$\alpha$ Null Mice Treated with Cisplatin and PPAR$\alpha$ Ligand**

The pattern of neutrophil infiltration after 3 days of cisplatin injection in wild-type mice is shown in Fig. 7, A–D. Neutrophil accumulation ($2 \pm 0.3$, 7 fields/mouse kidney) was rarely seen in saline- or WY-treated mice (Fig. 7, A and C); however, an
increase in the number of neutrophils was observed in the cortex and outer medulla of cisplatin-treated mice (35 ± 1.6, 7 fields/mouse kidney) as shown in Fig. 7B. Pretreatment with PPARα ligand significantly reduced the number of neutrophils present in cisplatin-treated mice (3 ± 0.6, 7 fields/mouse kidney) both in the cortex and the outer medullary region of the kidney (Fig. 7D). In contrast, neutrophil accumulation seen after 3 days of cisplatin injection (Fig. 7F) was not reduced in PPARα null mice treated with WY and cisplatin (Fig. 7H). The inhibition of neutrophil accumulation as well as the inhibition of cytokine/chemokine expression by the use of PPARα ligand underscore the potential therapeutic benefit of fibrates on the prevention of inflammation and amelioration of cisplatin-induced ARF.

Effect of Cisplatin and PPARα Ligand on NF-κB Binding Activity

NF-κB is a transcription factor that control genes involved a broad range of biological processes, including inflammation, immunity, and stress responses. Previous studies suggest that PPARα modulates inflammation via its interaction with the NF-κB signaling pathway (11–12). PPARα ligands can regulate NF-κB activity by reducing NF-κB DNA binding activity to target genes or by inducing the expression of inhibitor of NF-κB (IκBα). To evaluate the effects of cisplatin and PPARα ligand WY on NF-κB binding activity in the kidney, we prepared nuclear extracts from kidney tissue obtained from mice treated with saline (Fig. 8A, lane 2), cisplatin (lanes 3, 6, and 7), cisplatin+WY (lane 4), or WY (lane 5). NF-κB activity was determined by a binding assay using nuclear protein(s) and an oligonucleotide corresponding to the NF-κB consensus DNA labeled with -32P. As shown in Fig. 8, there were two DNA-protein complexes associated with NF-κB binding activity. The density of these two bands corresponding to NF-κB binding activity in kidney tissue was significantly increased by cisplatin treatment (P < 0.005, lane 3). The cisplatin-induced increased NF-κB binding activity corresponded to a new lower band not seen in nuclear extracts obtained from saline-treated mice. The density of this new band was significantly reduced by pretreatment with WY (lane 4). The binding specificity was demonstrated by the use of a cold unlabeled competitor DNA(s), NF-κB (homologous competitor), or AP-1 (heterologous competitor) oligonucleotides at 100-fold molar excess over the probe. The binding activity was completely abolished by cold unlabeled homologous competitor (lane 6). However, cold unlabeled heterologous competitor had no effect on NF-κB binding activity. Quantitative analysis of representative autoradiographs is presented in Fig. 8B.
DISCUSSION

In the present study, we have examined further the mechanisms by which PPARγ ligands protect renal function during cisplatin-induced ARF (27, 28, 33). Here, we demonstrate 1) that administration of cisplatin led to significant upregulation of cytokines TNF-α, IL-6, and IFN-γ; chemokines RANTES, MIP2, IP10, and MCP1; chemokine receptors CCR1 and CCR5; cell adhesion molecules ICAM-1,VCAM-1; and neutrophil accumulation in kidney tissue; 2) that pretreatment with PPARγ ligand WY afforded protection from cisplatin nephrotoxicity and significantly inhibited cisplatin-induced increased expression of TNF-α, RANTES, chemokine receptors CCR1 and CCR5, adhesion molecules VCAM-1 and ICAM-1, and prevented neutrophil accumulation in cisplatin-treated mice; 3) that PPARγ ligand did not prevent renal dysfunction or reduce cisplatin-induced neutrophil infiltration, or TNF-α and RANTES protein expression in kidney tissue from PPARγ null mice, findings that suggest that the cytoprotective effects of PPARγ ligand requires having an intact PPARγ gene for its observed anti-inflammatory effect during acute toxic renal injury; and 4) that cisplatin-increased NF-κB activity was reduced by the administration of PPARγ ligand, suggesting that the observed cytoprotection of PPARγ on cisplatin-induced gene expression of chemokines/cytokines is mediated, at least in part, by preventing NF-κB activation.

Role of Inflammation in Cisplatin-Induced Injury

There is increasing evidence that supports the role of inflammation in the pathophysiology of acute renal injury (4 –5, 18, 32). In particular, alterations in renal microvasculature with associated vasoconstriction have been shown to contribute to decreased renal blood flow early after cisplatin injection (29, 42). In addition, increased expression of endothelial cell adhesion molecules (26) and then subsequent infiltration of leukocytes have been also described in the model of cisplatin-induced ARF (36, 37). The increased accumulation of cytokines/chemokines by cisplatin is likely to trigger the expression of adhesion molecules on vascular endothelium and circulating leukocytes. Evidence for the pathogenetic role of adhesion receptor ICAM-1 was demonstrated by the administration of ICAM-1 monoclonal antibody, which led to decreased histological evidence of cisplatin-mediated tubular injury, medullary infiltration of inflammatory cells, as well as renal MPO activity (26).

Our studies also showed that cisplatin increased the expression of TNF-α, IL-6, and IFN-γ, which are proinflammatory cytokines involved a variety of inflammatory responses, including differentiation, maturation, and activation of inflammatory cells such as neutrophils, T cells, macrophages and natural killer cells. TNF-α, IL-6, and IFN-γ are also able to stimulate the expression of other cytokines and chemokines.
Fig. 7. Effects of cisplatin and PPARα ligand WY on histological evidence of renal neutrophil infiltration. PPARα wild-type (+/+) mice (A-D) or null (−/−) mice (E-H) were subjected to treatment with saline (A and E), WY (C and G), cisplatin (B and F), and WY + cisplatin (D and H). Kidney sections were immunostained for neutrophils as described in METHODS. Neutrophils appear as dark brown spots and are denoted by arrows. The sections are representative of at least 3 similar experiments.
Recent studies have demonstrated a role for TNF-α in mediating the inflammatory injury in cisplatin-induced ARF. Injection of cisplatin into mice led to upregulation of TNFR1 and TNFR2 mRNA levels in the kidney. The upregulation of TNFR2 but not TNFR1 was blunted in TNF-α-deficient mice, indicating ligand-dependent upregulation of TNFR2. To study the roles of each receptor, the authors administered cisplatin to TNFR1- or TNFR2-deficient mice. TNFR2-deficient mice developed less severe renal dysfunction and showed reduced necrosis and apoptosis and leukocyte infiltration into the kidney compared with either TNFR1-deficient or wild-type mice. Moreover, renal TNF-α expression, ICAM-1 expression, and serum TNF-α levels were lower in TNFR2-deficient mice compared with wild-type or TNFR1-deficient mice treated with cisplatin. Those results indicated that TNFR2 participates in cisplatin-induced renal injury in mice and may play an important role in TNF-α-mediated inflammation in the kidney in response to cisplatin. Our studies showing that the administration of a PPARα ligand reduced the expression of TNF-α and ICAM-1 and that this effect was accompanied by reduced neutrophil infiltration lend further support to those previous studies where reduced expression of ICAM-1 and TNFα were associated with decreased inflammatory response and amelioration of cisplatin induced nephrotoxicity.

A recent study has addressed the potential relationship between inflammation and the presence of apoptotic cell death in cisplatin-induced ARF. More specifically, the authors demonstrated that proinflammatory caspase 1 contributes to the pathogenesis of cisplatin-induced ARF. Caspase 1 activity increased before the development of severe renal dysfunction and caspase 1 null mice had less severe ARF and acute tubular necrosis. The cytoprotective effect of the caspase 1 null mice was attributed to reductions in proinflammatory cytokine IL-1β and renal neutrophil infiltration, as well as a reduction in caspase 3 activity and apoptosis (17). Although our current study did not evaluate the role of IL-1β or caspase 1 activation, our most recent studies have also demonstrated the antiapoptotic effect of PPARα ligand in cisplatin injury by reducing the expression and enzyme activity of proximal tubule mitochondrial endonuclease G and by decreasing the activity of caspase 3, which result in the amelioration of both proximal tubule cell apoptosis and necrosis (27, 34).

In the present study, we examined the effects of cisplatin on the expression of chemokines and chemokine receptors within the kidney. RANTES, MIP2, IP10, and MCP1 were significantly upregulated by cisplatin from day 2 to day 4, similar to previous published studies (13, 36). RANTES has been proposed as a major mediator of antigen-independent T lymphocyte activation and has been found expressed in T cells, macrophages, fibroblasts, vascular smooth muscle, endothelial cells, renal mesangial (30), and tubular epithelial cells (21). TNF-α can also transcriptionally stimulate RANTES expression via activation of NF-κB and p38 MAP kinase (23). Our studies also showed for the first time that mRNA levels of chemokine receptors CCR1 and CCR5 mRNA were upregulated by cisplatin. RANTES has been shown to bind to at least three different receptors, i.e., CCR1, CCR3, and CCR5.

CCR1 is the first chemokine receptor identified and expressed in neutrophils, monocytes, eosinophils, and T and B lymphocytes (19, 31). In a study using unilateral ureteral obstruction model in mice deficient for CCR1 or CCR5, Eis et al. (16) found that CCR1 but not CCR5 is required for leukocyte recruitment. Blockade of CCR1 with the CCR1-specific antagonist BX471 substantially reduced interstitial leukocyte accumulation and fibrosis in mice with glomerulosclerosis and nephrotic syndrome (41). Our observations demonstrating that the use of a PPARα ligand WY significantly inhibited cisplatin-induced upregulation of RANTES, and RANTES’s recep-
Role of PPARα in Modulating Inflammation in ARF

Our studies support the role of PPARα in preventing cisplatin-mediated inflammation in kidney tissue. Our results showing 1) that the protective effects of a PPARα ligand preventing the inflammatory response induced by cisplatin was only observed in wild-type mice and not in PPARα null mice and 2) that PPARα ligand prevented cisplatin-induced activation of NF-κB, as well as the increased production of known targets of NF-κB such as VCAM-1 and ICAM-1 suggest a protective role for PPARα in reducing not only cisplatin-induced tissue injury but also inflammation. Nevertheless, our studies cannot conclusively determine whether PPARα-mediated inhibition of NF-κB represents a direct mechanism by which the ligate prevented inflammation during cisplatin-induced ARF.

The first evidence for a role of PPARα in the control of the inflammatory response came from the observation that the inflammatory response induced by leukotriene B4 was prolonged in PPARα-deficient mice (15). Several other studies have confirmed the anti-inflammatory properties of PPARα. In clinical studies, administration of fibrates to patients with moderate hyperlipidemia decreased plasma concentrations of cytokines such as IL-6, TNF-α, IFN-γ, and acute-phase proteins such as fibrinogen and C-reactive protein (14). The PPARα repression of fibrinogen is due to negative interference of PPARα with the CAAT/enhancer binding protein pathway. In preventing cisplatin-mediated nephrotoxicity, therefore, in the cisplatin model of ARF we cannot conclusively determine whether PPARα ligands downregulate the inflammatory response that accompanies ARF.

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REFERENCES


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