PPARα ligand protects during cisplatin-induced acute renal failure by preventing inhibition of renal FAO and PDC activity

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Li, Shenyang, Pengfei Wu, Padma Yarlagadda, Nicole M. Vadjunec, Alan D. Proia, Robert A. Harris, and Didier Portilla. PPARα ligand protects during cisplatin-induced acute renal failure by preventing inhibition of renal FAO and PDC activity. Am J Physiol Renal Physiol 286: F572–F580, 2004. First published November 11, 2003; 10.1152/ajprenal.00190.2003.—Previous studies demonstrated that during cisplatin-induced acute renal failure, there is a significant reduction in proximal tubule fatty acid oxidation. We now report on the effects of peroxisome proliferator-activated receptor-α (PPARα) ligand Wy-14643 (Wy) on the abnormalities of medium chain fatty acid oxidation and pyruvate dehydrogenase complex (PDC) activity in kidney tissue of cisplatin-treated mice. Cisplatin causes a significant reduction in mRNA levels and enzyme activity of mitochondrial medium chain acyl-CoA dehydrogenase (MCAD). PPARα ligand Wy ameliorated cisplatin-induced acute renal failure and prevented cisplatin-induced reduction of mRNA levels and enzyme activity of MCAD. In contrast, in cisplatin-treated PPARα null mice, Wy did not protect kidney function and did not reverse cisplatin-induced decreased expression of MCAD. Cisplatin inhibited renal PDC activity before the development of acute tubular necrosis, and PDC inhibition was reversed by pretreatment with PPARα agonist Wy. Cisplatin also induced increased mRNA and protein levels of pyruvate dehydrogenase kinase-4 (PDK4), and PPARα ligand Wy prevented cisplatin-induced increased expression of PDK4 protein levels in wild-type mice. We conclude that PPARα agonists have therapeutic potential for cisplatin-induced acute renal failure. Use of PPARα ligands prevents acute tubular necrosis by ameliorating cisplatin-induced inhibition of two distinct metabolic processes, MCAD-mediated fatty acid oxidation and PDC activity.

Peroxisome proliferator-activated receptor-α; pyruvate dehydrogenase complex; fatty acid oxidation

WE DEMONSTRATED previously that during ischemia-reperfusion (I/R) and cisplatin-induced acute renal failure (ARF), there is a significant reduction in proximal tubule fatty acid oxidation (FAO) that leads to the accumulation of toxic fatty acid amphiphiles (23, 24, 27). Furthermore, our studies also showed that the reduction in FAO observed in kidney tissue during ARF occurs as a result of a direct inhibition of peroxisome proliferator-activated receptor-α (PPARα) DNA binding activity to its target genes and also by reduced expression of its tissue-specific coactivator PGC-1 (23). The use of PPARα ligands such as clofibrate, Wy-14643 (Wy), and etomoxir significantly ameliorated proximal tubule cell injury and renal function during I/R injury. These results underscore the importance of PPARα-mediated regulation of FAO as a potential therapeutic target that could be used in various models of ARF.

Previous studies in muscle tissue documented abnormalities in glucose homeostasis during ARF (4). The potential presence of similar metabolic abnormalities in kidney tissue during ARF has not been explored. PPARα has been recently shown to modulate glucose metabolism via regulation of pyruvate dehydrogenase complex (PDC) activity in skeletal muscle tissue (11). Regulation of the activity of PDC is an important component of the regulation of glucose homeostasis (8). PDC is a multienzyme complex that catalyzes the conversion of pyruvate to acetyl-CoA. This reaction is the first irreversible step in the oxidation of carbohydrate-derived carbon and regulates the entry of carbohydrate into the tricarboxylic acid cycle. PDC activity is regulated by reversible phosphorylation and dephosphorylation reactions catalyzed by an intrinsic kinase and phosphatase (8, 11, 15, 22). Phosphorylation of E1 catalytic subunits of PDC by pyruvate dehydrogenase kinase (PDK) causes inactivation of the enzyme, whereas pyruvate dehydrogenase phosphatase (PDP) removes phosphate and returns the enzyme to its active form (PDCa) (22). The relative activities of the PDPs and PDKs determine the proportion of the complex in its active form. There are four known isoforms of PDKs, which differ significantly in their tissue distribution, specific activity, and sensitivity to their effectors (26, 30). To date, PDK4 and PDK2 are the two most abundant PDK isoforms expressed in rodents and human kidney tissue (29, 31, 33).

In the present study, we examined the effects of PPARα ligand Wy on cytoprotection in the toxic model of cisplatin-induced ARF. More specifically, we examined the effects of cisplatin on renal function and histological parameters of proximal tubule injury in the absence and presence of Wy. To address the potential mechanisms of protection by PPARα ligands, we also examined the effects of this ligand on medium chain acyl-CoA dehydrogenase (MCAD)-mediated FAO, PDC activity, and mRNA and protein levels of PDK isoforms in kidney tissue. Our studies corroborate previous observations that cisplatin-induced ARF is accompanied by significant attenuation of mRNA and enzyme activity of mitochondrial FAO enzyme MCAD (25). The use of PPARα ligand Wy ameliorated both proximal tubule cell injury and kidney function in the PPARα wild-type mice but not in the PPARα null mice.

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Increased mRNA and enzyme activity of renal FAO enzyme MCAD accompanied this protective effect. In addition, we observed that cisplatin induced a profound and sustained inhibition of PDC activity detected on the second day after cisplatin injection and before the development of acute tubular necrosis (ATN). PDC inhibition was accompanied by increased expression of protein and mRNA levels of renal mitochondrial PDK4 isoform. Pretreatment with PPARα ligand WY reversed the inhibition of PDC activity by cisplatin and prevented the observed increased expression of kidney PDK4 protein levels in cisplatin-treated mice. Our results demonstrate for the first time in kidney tissue that cisplatin-induced ARF is accompanied by the presence of significant metabolic changes, which include inhibition of MCAD-mediated FAO, as well as the inhibition of glucose metabolism via inhibition of PDC activity. Pretreatment with PPARα ligand WY reversed the inhibition of MCAD and PDC activities and correlated with protection of kidney function. Further studies are needed to delineate the nephron segments, and the mechanisms by which PPARα activation modulate substrate oxidation, and protect kidney function during ARF.

METHODS

In vivo model of cisplatin-induced ARF. Male sv129 mice ~8–10 wk old, weighing 25 to 30 g, were assigned to treatment groups (8 animals/group). Animals received single intraperitoneal doses of vehicle (saline) or cisplatin (20 mg/kg body wt). After treatment, the animals were killed and the kidney tissue was 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. To investigate the effects of PPARα activation, mice were fed the WY-containing diet for 2 wk. For histopathological evaluation, kidneys were collected in 10% neutral buffered formalin. Blood urea nitrogen (BUN) and creatinine were measured by an enzymatic colorimetric assay as previously described (5, 9).

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 Guiding Principles in the Care and Use of Laboratory Animals.

Histopathological alterations. We evaluated histopathological alterations in the kidneys 4 days after the mice were treated with cisplatin with or without WY. Kidneys were bisected, fixed in 3.7% phosphate-buffered neutral formaldehyde, dehydrated with serial alcohols, and embedded in paraffin. We stained 3-μm-thick paraffin sections with hematoxylin and eosin and a periodic acid-Schiff (PAS) method (20). The 12 morphological features described by Soley and co-workers (28) were evaluated in a masked fashion: leukocyte accumulation in the vasa recta: tubular necrosis (presence of necrotic cells, apparently denuded areas of tubular basement membrane, or ruptured tubular basement membranes); tubular regeneration; mitotic figures in tubular cells; dilatation of Bowman’s space with retraction of the glomerular tuft (“acute glomerular ischemia”); loss of PAS-positive tubular brush border; “tubularization” of the parietal epithelium of Bowman’s capsule; tubular casts; interstitial inflammation; interstitial edema; tubular dilatation; and prominence of the juxtaglomerular apparatus. We graded the morphological changes on a scale from 0 to 2 where 0 = none; 1/2 = minimal; 1 = mild; 1/2 = moderate; and 2 = marked (14).

RNA isolation. Mice were killed following previously described experimental conditions, and the kidney tissue was 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.

RT-PCR. Total RNA extract was treated with 1 U of RQ1 RNase-free DNase (Promega) per microgram of total RNA at 37°C for 1 h. Reverse transcription 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−RT (Invitrogen Life Technologies). Subsequently, RT was inactivated by incubation at 70°C for 15 min, followed by treatment with 1.2 U of 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 containing the 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. Amplification was performed using the following primer pairs for 25 cycles (denaturation at 94°C for 30 s, annealing at 57°C for 25 s, and extension at 72°C for 30 s): PDK4 sense (5′-ACCTTCTACTCGGATG-3′) and antisense (5′-CCTCCTCGGTCA-GAATCTT-3′) primers, GAPDH sense (5′-AATCTTGGCATTGTTG-GGAAGG-3′) and antisense (5′-AGATCCGACGACCAA-3′) primers, and MCAD sense (5′-GTACCCGTTCCTCTCATCA-3′) and antisense (5′-CTGCGCAAGAATAACC-3′) primers. The sense primer was end-labeled using [γ-32P]ATP (PerkinElmer) and T4 polynucleotide kinase (New England Biolabs). Five microliters from the PCR reactions were loaded on a 4% acrylamide gel. The gels were analyzed on a 445 SI PhosphorImager with ImageQuant (Molecular Dynamic), and then subjected to autoradiography. Results are presented as the ratio of the signal for PDK4 or MCAD band to that of the GAPDH signal.

Western blotting. PDK2 and PDK4 protein levels were estimated by Western blot analysis of mouse kidney tissue extracts obtained from several experimental conditions, as described previously (32).

PDC activity. Mice were killed after experimental conditions, and the kidney tissue was rapidly snap-frozen in liquid nitrogen and stored at −75°C. Frozen kidney tissue was extracted as described previously by Goodwin et al. (6) with modification. In brief, 50 mg tissue were homogenized in 0.5 ml of homogenization buffer with a Teflon homogenizer (7–10 strokes). The resulting homogenate was subsequently centrifuged at 10,000 g at 4°C for 10 min to remove tissue debris, and the supernatant was removed, placed on ice, and enzyme activity was assayed immediately. To determine the fraction of PDC in the active form (PDCa), tissue was extracted using a homogenization buffer containing 30 mM K-HEPES (pH 7.5), 3% Triton X-100, 5 mM EDTA, 10 mM EGTA, 10 mM DCA, 50 mM KF, 2% bovine serum, 0.01 mM TPCK, 10 μg/ml trypsin inhibitor, 1 μM, and 5 mM DTT. Under these conditions, both PDK and phosphatase were inhibited. To determine the total activity of PDC (PDCt), tissue was extracted under conditions where PDP was stimulated (and also with addition of exogenous PDP) and PDK was inhibited, using a homogenization buffer consisting of 30 mM K-HEPES (pH 7.5), 3% Triton X-100, 2% bovine serum, 0.01 mM TPCK, 10 μg/ml trypsin inhibitor, 1 μM leupeptin, 5 mM EDTA, 20 mM DCA, 15 mM MgCl2, and 1 mM CaCl2. Enzyme activity assays of PDC in the above extracts were determined as described previously (20) with a SPECTRAMax 190 Micro plate Spectrophotometer (Molecular Devices, Sunnyvale, CA).

MCAD activity. MCAD activity assay was performed in kidney tissue extracts following protocol previously described by Lehman et al. (17) with minor modifications. Kidneys were homogenized in cold 100 mM HEPES with 0.1 mM EDTA (pH 7.6). The homogenates were then centrifuged briefly at 4°C and the MCAD activity was measured immediately on the supernatant at 37°C. Two microliters of supernatant were added to 200 μl of reaction solution of 100 mM HEPES buffer (pH 7.6), 0.1 mM EDTA, 200 μM ferricenium hexafluorophosphate, 0.5 mM sodium tetraphosphate, and 50 μM octanoyl-CoA. The absorbance decrease at 500 nm in the ferricenium ion was determined by SPECTRAMax microplate spectrophotometer (Molecular Devices) over the initial 60-s period. The values were corrected by subtracting the background absorbance of a tissue blank, measured
in the absence of octanoyl-CoA in the reaction solution. Results are presented as means ± SE of MCAD activity relative to that obtained for control mice, which was set arbitrary as 100% in each experiment and was calculated from at least three independent experiments.

Statistics analysis. Results are presented as means ± SE. Statistical analysis was performed using unpaired Student’s t-tests. A P value of <0.05 was considered to be statistically significant.

RESULTS

Effect of PPARα ligand WY on body weight in PPARα wild-type (+/+ ) and null (−/− ) mice. Mean body weight was significantly lower in PPARα wild-type mice fed WY-containing diet compared with wild-type mice that received a similar regular chow diet. The PPARα wild-type mice lost 10% of their body weight after receiving WY diet for 2 wk. On the contrary, the PPARα null mice fed a WY-containing diet gained 3% of their body weight compared with null mice receiving a regular chow diet. The loss of body weight in PPARα wild-type mice fed WY is consistent with previous reports and is likely related to ligand-mediated increased FAO by liver and kidney tissue (1).

PPARα ligand WY protects kidney function during cisplatin-induced ARF in PPARα wild-type mice but not in PPARα null (−/− ) mice. Kidney function was monitored for 4 days after intraperitoneal injection of saline or cisplatin, by measuring BUN and serum creatinine. Figure 1, A and B, presents the changes in BUN and creatinine seen in PPARα wild-type mice treated with and without WY ligand in the absence (Control) and presence of cisplatin.

Comparison of the renal function between PPARα wild-type mice fed for 2 wk with either regular diet or 0.1% WY-containing diet did not show differences in BUN and creatinines when treated with a single injection of vehicle alone.
Three of four kidneys from mice treated with WY also had occasional mitotic figures evident in the tubular epithelium (score = 0.5). All four mice treated with cisplatin alone had moderate to marked tubular necrosis and loss of the epithelial cell brush border, numerous casts, and three of four had regenerative tubular changes manifest as flattened, hypereosinophilic epithelial cells. There was a minimal infiltrate of leukocytes in the vasa recta of one mouse and a mild infiltrate in one mouse (data not shown), but none had tubular epithelial cell mitoses. All four mice treated with WY together with cisplatin were readily distinguishable from the mice treated with cisplatin alone because they had only minimal epithelial cell necrosis, no or only minimal loss of the brush border, and no casts. The kidneys from mice receiving cisplatin and WY were distinguishable histologically from those treated with only WY by the absence of epithelial cell mitotic figures. An example of a kidney from a control (untreated) mouse and typical mice treated with WY, cisplatin, and cisplatin + WY is shown in Fig. 2. Also shown in this figure is the study of the effects of WY ligand on PPARα null mice. A histological section from a PPARα−/− mouse demonstrates the subtle loss of PAS-positive brush border in a few tubules, whereas a histological section of a PPARα−/− mouse treated with WY and cisplatin shows necrosis of the tubular epithelium. Again, these histopathological alterations are consistent with the results shown in Fig. 1 on renal function. Altogether, our data suggest that PPARα ligand WY protects PPARα wild-type mice and not PPARα null mice from cisplatin-induced ARF likely by a mechanism dependent on having an intact PPARα gene.

Effects of PPARα ligand WY on the expression of MCAD. To determine the mechanisms by which PPARα ligand protects renal function and ameliorates histological parameters in cisplatin-induced ATN, we examined the effects of cisplatin and WY ligand on renal MCAD activities of both PPARα wild-type and null mice. As shown in Fig. 3, A-C, by representative autoradiograms and PhosphorImaging analysis, cisplatin caused a progressive decline in the mRNA expression of MCAD, a rate-limiting enzyme in the metabolism of medium chain fatty acids by mitochondria in kidney tissue. At day 4 of renal failure, there was a 63% inhibition of MCAD expression compared with control mice (P < 0.005). In PPARα null mice (see Fig. 3B), cisplatin at day 4 had a similar effect on MCAD mRNA levels. On day 4 following cisplatin injection, there was a 65% inhibition of MCAD activity (P < 0.005). Pretreatment with WY ligand reversed cisplatin-induced inhibition of renal MCAD activity in wild-type mice, but this effect was not observed in the PPARα null mice (see Fig. 3C). These results are similar to our previously published observations in which the use of PPARα ligands etomoxir and clofibrate resulted in upregulation of renal FAO during I/R. Therefore, again these observations further corroborate the cytoprotective role of PPARα ligands on renal function during ARF and further underscore the importance of mitochondrial FAO on the preservation of structure and function of the proximal tubule during ARF.

Effects of PPARα ligand WY on the enzyme activity of MCAD. Because PPARα ligand WY prevented cisplatin-induced reduction of MCAD mRNA levels in the PPARα wild-type mice but not in the PPARα null mice, we next examined the effects of cisplatin and WY on MCAD enzyme activity. As shown in Fig. 4, cisplatin on day 4 caused a profound decline in the enzyme activity of renal MCAD in both PPARα wild-

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Table 1. Grading of lesions in kidneys after cisplatin administration with or without Wy-14643 pretreatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular Necrosis</th>
<th>Tubular Regeneration</th>
<th>Mitoses</th>
<th>Brush Border Loss</th>
<th>Casts</th>
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<td>Control</td>
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<td>0</td>
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<td>Means ± SD</td>
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<tr>
<td>Wy-14,643 treated</td>
<td>0.5 ± 0.5</td>
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<td>Means ± SD</td>
<td>0.5 ± 0.5</td>
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<tr>
<td>Cisplatin</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Means ± SD</td>
<td>1.5 ± 0.5</td>
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</tr>
<tr>
<td>Cisplatin + Wy-14,643</td>
<td>0.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
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<tr>
<td>Means ± SD</td>
<td>0.5 ± 0.5</td>
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Grading system is that of Solez et al. (28), and scoring system is that of Kelleher et al. (14) where 0 = none, 0.5 = minimal, 1 = mild, 1.5 = moderate, and 2 = marked.
Cisplatin-induced inhibition of PDC activity in toxic acute renal failure

Fig. 2. Histopathological alterations in mice treated with PPARα ligand and cisplatin: PPARα +/+. A histological section from a control (untreated) kidney (A) discloses a normal glomerulus and tubules, whereas a typical kidney from a mouse treated with WY (B) demonstrates an epithelial cell mitosis (large arrowhead) and loss of the PAS-positive brush border in some tubules (arrow). A typical kidney from a mouse treated with cisplatin (C) shows extensive necrosis of the tubular epithelium (*) and the tubules shown in C, left, are necrotic and similar in appearance to that denoted with the arrow. A section from a typical cisplatin + WY-treated kidney (D) has only a rare necrotic epithelial cell (large arrowhead) and loss of the PAS-positive brush border in some tubules (arrow). PPARα −/−: A: histological sections from a PPARα null mice treated with WY also demonstrates loss of the PAS-positive brush border in a few tubules (see arrow). B: kidney sections from PPARα −/− mice treated with cisplatin and WY showed marked tubular necrosis of the tubular epithelium (*). All sections were stained with PAS reagent and photographed at the same magnification (magnification bar = 50 μm).

Pretreatment with WY prevented cisplatin-induced reduction of renal MCAD activity in PPARα wild-type mice. In contrast to the effects of WY on PPARα wild-type mice, pretreatment with WY did not affect cisplatin-induced reduction of MCAD activity in PPARα null mice. These data further support our previous results showing that the protective effect of PPARα ligand WY on mRNA levels and activity of FAO enzyme MCAD were dependent on having an intact and functionally active PPARα gene.

Cisplatin-induced inhibition of PDC activity is prevented by PPARα ligand WY. Previous studies support the notion that ARF is accompanied by abnormal glucose oxidation in muscle tissue (4, 19). There is no experimental evidence that renal pathways involved in glucose homeostasis are affected during ARF. Therefore, we examined first the effect of cisplatin-induced ARF on PDC activity. Cisplatin led to a rapid inhibition of mitochondrial PDC activity detected within the first 48 h after cisplatin administration (P < 0.001; results not shown). This effect was sustained, and by day 4 as shown in Fig. 5, A and C, there was more than 70% inhibition of mitochondrial PDC activity by cisplatin (**P < 0.0005). Pretreatment of PPARα wild-type mice with WY prevented cisplatin-induced inhibition of PDC activity as shown in Fig. 5C. In addition, WY treatment although alone decreased the fraction of both PDCa and PDCt in kidney tissue did not significantly affect PDC activity measured as a percentage of PDCa/PDCt as shown in Fig. 5C. These studies suggest that in addition to preventing cisplatin inhibition of FAO, WY also to some extent restores the capacity for the kidney to oxidize glucose. Therefore, our studies further support the role of PPARα as a master regulator of substrate oxidation in kidney tissue.

Effects of PPARα ligand and cisplatin on the expression of PDK4 mRNA levels. There are recent reports that PPARα may play a role in the regulation of glucose metabolism (35). Previous studies showed that increased expression and activity of PDK account for the inhibition of PDC activity in pathophysiological states such as starvation, diabetes, and hyperthyroidism (10, 33). To determine the mechanisms by which PDC activity is inhibited by cisplatin, we examined the effects of cisplatin and WY ligand on PDK4 mRNA levels of both PPARα wild-type and null mice. As shown in Fig. 6, A−C, by representative autoradiograms and PhosphorImaging analysis, cisplatin caused a progressive increase in the mRNA expression of PDK4 mRNA levels. As shown in Fig. 6, A and C, in PPARα wild-type mice at day 4 of renal failure, there was a profound upregulation (8-fold) of PDK4 mRNA levels compared with control mice (**P < 0.005). In PPARα null mice (see Fig. 6, B and C), cisplatin at day 4 had only a modest effect, causing a 2.8-fold stimulation of PDK4 mRNA levels.
Pretreatment with WY ligand also led to a fivefold increase in PDK4 mRNA levels in wild-type mice, but this effect was again significantly lower (only 2-fold increase) in the PPARα/H9251 null mice (see Fig. 6C). We next examined the effects of WY on cisplatin-induced increased expression of PDK4. As shown in Fig. 6A and C, cisplatin was unable to further increase PDK4 mRNA expression compared with cisplatin-treated null mice. In contrast, in WY-fed PPARα null mice cisplatin significantly increased PDK4 mRNA levels in kidney tissue of PPARα null mice compared with control, cisplatin-, or WY-treated null mice.

**Effects of cisplatin and WY on the expression of renal PDK4 protein.** Because changes in PDK4 mRNA levels did not correlate with changes in PDC activity, in the next series of studies we investigated the effects of cisplatin and WY on the protein levels of PDK4 using Western blot analysis of mouse kidney tissue. As shown in Fig. 7 by a representative autoradiogram and by densitometric quantification, cisplatin induced a profound upregulation (8-fold) of PDK4 protein levels in wild-type mice. In WY-treated wild-type mice, as well as in WY-treated mice that received cisplatin, PDK4 protein was not expressed. We conclude that increased protein levels of PDK4 induced by cisplatin in the wild-type mice represent one of the mechanisms by which PDC activity is inhibited and that the inhibition of the expression of PDK4 protein by PPARα ligand WY helps preserve PDC activity during ARF.

**DISCUSSION**

In the present studies, we extended previous observations made in the I/R injury model of ARF. We demonstrated herein (1) similar to I/R injury, kidney injury from cisplatin-induced ARF is accompanied by decreased expression and inhibition of enzyme activity of mitochondrial FAO enzyme MCAD; (2) pretreatment with a specific PPARα ligand protected renal function, and this protection in renal function was substantiated by histopathological examination that demonstrated amelioration of cisplatin-induced acute tubular necrosis, loss of brush border, and cast formation; (3) pretreatment with PPARα ligand also reversed reduced expression and enzyme activity of kidney MCAD induced by cisplatin; (4) the persistent inhibition of FAO, and lack of protection of renal...
function in PPARα null mice treated with cisplatin and WY, further underscores the importance of having an intact PPARα gene in the observed cytoprotective effect of PPARα ligands during ARF (23, 24). These results corroborate the importance of having a preserved renal PPARα-modulated FAO pathway in the observed protection of renal function during ARF.

Our histopathological analysis of mice treated with PPARα ligand WY also revealed the presence of proliferation of a few isolated proximal tubular cells, and minimal loss of brush border, effects that were not accompanied by significant changes in renal function. The significance of these findings is unknown but perhaps represents a toxic effect of this ligand on kidney tissue. The use of more potent PPARα ligands that could be given at the time of cisplatin injection in future studies should allow us to avoid these side effects. In addition, our studies cannot completely rule out the possibility of a potential effect of the PPARα ligand on preconditioning, an effect that can protect kidney tissue from further injury.

Because the observed protection in kidney function in PPARα wild-type mice that received a PPARα ligand likely involves modification of various metabolic pathways, other than FAO in kidney tissue, we also examined the effect of PPARα ligand on PDC activity. Abnormalities in glucose metabolism have been previously described in skeletal muscle of rats subjected to ARF (4). Carbohydrate metabolism is deranged in ARF as a result of impaired insulin-mediated actions. Previous studies suggest that insulin resistance accounts for one of the hormonal abnormalities involved in the deranged muscle protein catabolism in ARF. Those studies demonstrated decreased insulin-mediated glucose uptake, glycogen synthesis, and glucose oxidation in the muscle of acutely uremic rats (19). Clark and Mitch (4) suggested that the abnormal net protein degradation in ARF may be linked to the altered carbohydrate metabolism and might be a consequence of defective glucose oxidation. In the present study, we examined the effects of cisplatin-induced ARF on PDC activity, the enzyme that catalyzes the conversion of pyruvate to acetyl-CoA in the mitochondria. Our studies demonstrate the inhibition of PDC activity as early as 48 h after cisplatin injection, a time point at which we could not detect significant changes in BUN or creatinine, or histopathological parameters of tubular injury. In addition, prior administration of PPARα ligand WY reversed cisplatin-induced inhibition of PDC activity. The significance of this observation is great, because preservation of PDC activity perhaps represents an important metabolic adaptation of distal nephron segments to the lack of energy production during ARF.

The differential regulation of PDK and phosphatase is the key to the overall regulation of the activity of the PDC. Three specific serine residues in each α-subunit of the E1 domain, namely, site 1 Ser264, site 2 Ser271, and site 3 Ser203, are subject to ATP-dependent phosphorylation and inactivation by PDKs (8). PDP dephosphorylates these three serine residues and reactivates PDC (3, 18). Mammalian PDK isoenzymes differ in their catalytic activity, responsiveness to modulators like NADH and acetyl-CoA, and tissue-specific expression. PDK1 is present mostly in the heart, whereas PDK2 is found in most tissues. PDK3 is predominantly expressed in testis, whereas heart, skeletal muscle, and kidney have the highest amount of PDK4 (2, 7, 31). In pathophysiological states such as starvation, diabetes, and hyperthyroidism (10, 29, 34), PDC activity is reduced and mRNA and protein levels of PDK4 are increased. Our studies showing a temporal relationship between cisplatin-induced inhibition of PDC activity and increased expression of renal PDK4 protein suggest a similar mechanism of regulation of PDC activity. In addition, our data also suggest that increased phosphorylation of PDC by free PDK4 could be a potential mechanism that accounts for the observed inhibition of PDC activity during ARF. However, our current data cannot conclusively establish a cause-effect relationship between these two metabolic abnormalities, because we also observed decreased PDC activity in cisplatin-treated PPARα null mice and these mice did not express PDK4 protein when treated with cisplatin. Therefore, other potential pathways by which PDC activity could be downregulated during the study.
cisplatin could include decreased expression of PDPs, or changes in the levels of NAD, NADH. In fact, recent studies suggest that starvation and streptozotocin-induced diabetes cause decreases in PDP2 mRNA abundance, PDP2 protein amount, and PDP activity in rat heart and kidney. Refeeding and insulin treatment effectively reversed these effects of starvation and diabetes, respectively. Those findings indicate that opposite changes in expression of specific PDK and PDP isoenzymes contribute to hyperphosphorylation and therefore inactivation of the PDC in heart and kidney during starvation and diabetes (12). More studies will be needed to completely clarify all the mechanisms that contribute to renal PDC inhibition during ARF.

Our studies also suggest that by day 4 cisplatin inhibits gene expression of enzymes associated with FAO and pyruvate oxidation, two important metabolic substrates for the generation of acetyl-CoA, a three-carbon molecule that is used by normal kidney tissue via the TCA cycle for the generation of energy in the form of ATP. Metabolic abnormalities resulting from the inhibition of these two important sources of energy are likely to further contribute to the catabolic state of ARF and perhaps explain the metabolic need for increased protein degradation during ARF. Therefore, increased oxidation of amino acids by kidney tissue, as a maladaptive mechanism, is likely to provide the substrate needed for the generation of acetyl-CoA and energy production via the TCA cycle in the setting of decreased energy production. Of interest, a recent study (13) demonstrated that inhibition of protein degradation using a specific proteasome inhibitor results in cytoprotection during I/R injury to the kidney.

In the present study, we did not examine whether PPARα ligand had an effect on the apoptotic response to cisplatin. In preliminary studies not shown here, we only found positive TUNEL staining in the kidneys of PPARα wild-type mice treated with cisplatin, which represented less than 5% of cellular injury seen in the corticomedullary junction, with necrosis representing the major form of cell death. These similar findings have been previously reported by other investigators (21). Of interest, our most recent studies done in
proximal tubule cells in culture showed that PPARα ligand inhibits caspase 3 activation by cisplatin, and this results in amelioration of proximal tubule cell death (25), suggesting that PPARα-mediated inhibition of the caspase cascade might also represent a mechanism of cytoprotection.

In conclusion, our results clearly indicate the ability of PPARα ligand to ameliorate cisplatin-induced ARF. In addition, our studies further support the role of inhibition of MCAD-mediated FAO and inhibition of PDC activity in the pathogenesis of cisplatin-induced ARF. Further studies are needed to localize the nephron segment(s) where these abnormalities in PDC and FAO take place in the kidney, to further examine the cellular mechanisms of substrate inhibition, and to determine whether PPARα ligands have similar effects on these cellular pathways, as our studies suggest it is the case in this in vivo study.

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