Reduced kidney lipoprotein lipase and renal tubule triglyceride accumulation in cisplatin-mediated acute kidney injury

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We and others have documented the presence of metabolic abnormalities that coexist with the development of acute kidney injury (AKI) (5, 24, 26–29, 39–41). More specifically, hyperglycemia and insulin resistance have been associated with increased mortality during AKI in the intensive care unit setting (5). We have reported the accumulation of free fatty acids in serum, hyperglycemia, and hyperinsulinemia, in addition to the accumulation of neutral lipids in the proximal tubule in mice treated with cisplatin (40, 41). These findings support the presence of a systemic effect of cisplatin on lipid metabolism, a phenomenon previously documented in rats treated with cisplatin (1), as well as in patients surviving testicular cancer as a long-term complication of platinum-based chemotherapy (33). Currently, the mechanisms involved in cisplatin-mediated intracellular accumulation of triglycerides (TG) and its relationship to nephrotoxicity are not clear.

While adipose tissue has a unique capacity to store excess fatty acids in the form of TG in lipid droplets, non-adipose tissue such as renal tubular cells have a limited capacity for such storage of lipids (55). In hyperlipidemic states, accumulation of excess lipid in non-adipose tissues leads to cell dysfunction and/or cell death, a phenomenon known as lipotoxicity (49). Besides being important for storage and subsequent release of fatty acids, research over the last decade has shown that white adipose tissue also has an important endocrine function (2). Altered secretion of adipocyte-derived proteins contributes to the increased metabolic and cardiovascular risk found in obesity (15, 48). There are several pathways that allow the uptake of circulating lipids into cells. Cell surface receptors mediate the uptake of whole lipoproteins. The hydrolysis of circulating TG bound to chylomicrons and very low-density lipoproteins (VLDL) is catalyzed by the enzyme lipoprotein lipase (LPL), which is anchored to the capillary endothelium via heparin sulfate proteoglycans and is a key determinant of cellular fatty acid uptake (18, 54). LPL is expressed at high levels in tissues that depend on fatty acids as fuel (heart, skeletal muscle, kidney cortex) or synthesize fats for storage or secretion (adipose tissue, mammary tissue). The activity of LPL is regulated by various mechanisms (31, 54). In the present study, we examined the effects of cisplatin on the expression of kidney LPL activity and on the expression of several regulators of kidney LPL activity, including angiopoietin protein-like 4 (Angptl4) (20, 25), glycosylphosphatidylinositol-anchored-HDL-binding protein (GPIHBP1), and lipase maturation factor 1 (Lmf1). We demonstrate that J) cisplatin

CISPLATIN IS AN EFFECTIVE CHEMOTHERAPEUTIC AGENT, BUT THE DEVELOPMENT OF NEPHROTOXICITY IS A MAJOR LIMITING FACTOR ASSOCIATED WITH ITS USE (3, 9, 35). IN THE KIDNEY, CISPLATIN ACCUMULATES IN THE PROXIMAL TUBULE AND ACTIVATES COMPLEX SIGNALING PATHWAYS, LEADING TO TUBULAR CELL DEATH (11, 10, 42). IN ADDITION, CISPLATIN ACTIVATES A ROBUST INFLAMMATORY RESPONSE COMPANIONED BY VASCULAR ENDOTHELIAL DAMAGE TO KIDNEY TISSUE, WHICH ULTIMATELY CONTRIBUTES TO ISCHEMIC INJURY, REDUCED GLOMERULAR FILTRATION RATE, AND ACUTE ORGAN FAILURE (22, 36).

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significantly reduces epididymal white adipose tissue mass, leading to focal necrosis and increased lymphocytic infiltration; 2) cisplatin administration reduces the expression levels and enzyme activity of LPL in epididymal white adipose tissue as well as in kidney tissue; 3) cisplatin administration increases Angptl4 mRNA and protein levels in kidney tissue and reduces mRNA levels of kidney GPIHBP1 and Lmf1; and 4) treatment with a peroxisome proliferator-activated receptor-α (PPARα) ligand prevents cisplatin-mediated reduced expression and activity of LPL in kidney tissue. Our studies suggest that changes on the expression of kidney Angptl4, GPIHBP1, and Lmf1 gene expression may represent a cellular mechanism(s) involved in cisplatin-mediated regulation of kidney LPL.

**METHODS**

**Animal Model of Cisplatin-Induced AKI**

Experimental AKI was induced in 8- to 0-wk-old male mice (strain Sv129) using cisplatin administration. The animals used in these studies were housed at the Veterinary Medical Unit at the Central Arkansas Veterans Healthcare System (Little Rock, AR). When appropriate, animals were painlessly euthanized according to methods of euthanasia approved by the Panel on Euthanasia of the American Veterinary Medical Association. Our animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Central Arkansas Veterans Healthcare System.

Animals were maintained on standard chow, and, as indicated, a group of animals was fed with a special diet containing WY-14643 (0.1%; WY) for 10 days before cisplatin administration. Cisplatin was administered by a single intraperitoneal injection of 20 mg/kg body wt. After the induction of renal failure, the animals were returned to their cages and allowed free access to food and water.

**Cell Culture and Differentiation**

To further examine the cellular effects of cisplatin on LPL activity in adipose tissue, we performed in vitro studies using an adipocyte cell line. 3T3-F442A cells were obtained from Dr. Howard Green (Harvard Medical School, Boston, MA). Cells were maintained in DMEM (GIBCO BRL) supplemented with 10% calf serum. For experiments, cells were grown to 70% confluence and stimulated to differentiate in DMEM containing 10% fetal bovine serum and 100 nM insulin for 14 days. Cells were treated with saline or the specified concentration of cisplatin for 24 h.

**Gene Expression Studies**

LPL, Angptl4, GPIHBP1, and Lmf1 mRNA levels were determined by quantitative real-time RT-PCR. Total RNA was extracted from cells or mouse kidney tissue and treated with RNase-free DNase before RT reaction. Real-time PCR was carried out using the StepOnePlus real-time PCR system (Applied Biosystems) with iTaqSYBR Green Supermix with Rox (Bio-Rad). In each experiment, triplicates of 50 ng cDNA (total RNA equivalent) of samples were amplified in a 20-μl reaction. Specificity of the amplified product was confirmed by melting curve analysis and agarose gel electrophoresis. For relative quantification, a standard curve was generated from a sample of LPL mRNA expressed in the negative control cell line. 3T3-F442A cells were obtained from Dr. Howard Green (Harvard Medical School, Boston, MA). Cells were maintained in DMEM (GIBCO BRL) supplemented with 10% calf serum. For experiments, cells were grown to 70% confluence and stimulated to differentiate in DMEM containing 10% fetal bovine serum and 100 nM insulin for 14 days. Cells were treated with saline or the specified concentration of cisplatin for 24 h.

**Protein Two-Dimensional Separation and Blotting**

The cytoplasmic protein fraction was extracted from mouse kidneys using cell lysis buffer containing protease inhibitors (Sigma). Protein concentration was determined using a Bradford protein assay reagent (Bio-Rad). Protein samples (100 μg) were separated by two-dimensional (2D) gel electrophoresis [sievelectric focusing (IEF) as the first dimension and SDS-PAGE as the second]. Protein was introduced in 125 μl of rehydration buffer (8 M urea, 2% CHAPS, 40 mM DTT, and 0.2% Biolyte). IEF was performed in a PROTEAN IEF Cell (Bio-Rad), following the voltage-gradient protocol as follows: S1, 250 V for 15 min; S2, 300 V for 15 min; S3, 500 V for 30 min; S4, at 4,000 V for 12,000 V-hours; and S5, 4,000 V for 2 h. The strips were first equilibrated for 15 min in 0.375 M Tris-Cl buffer (pH 8.80 containing 130 mM DTT, 6 M urea, 20% glycerol, and 2% SDS). A second equilibration step was carried out for 15 min in equilibration buffer containing 135 mM iodoacetamide instead of DTT. IEF strips were electrophoresed in 12% Invitrogen precast gels to separate and blotted onto nitrocellulose membranes.

**Western Blotting and Quantification**

Samples separated by 2D electrophoresis were blotted onto nitrocellulose membranes (Schleicher & Schuell). The membrane was blocked in 5% milk in TBS+0.1% Tween for 1 h at room temperature and incubated with Angptl4 antibody at 4°C overnight at a dilution of 1:1,000. We used a rabbit polyclonal antibody raised against full-length rat Angptl4. Secondary antibody was HRP-linked polyclonal anti-rabbit (1:5,000, Cell Signaling), incubated for 1 h at room temperature. Before and after secondary antibody incubations, membranes were washed four times with TBST to remove nonspecific binding. Chemiluminescence detection was performed using a SuperSignal Femto kit (Pierce, Rockford, IL). The blots were stripped and reprobed with actin antibody for protein load normalization control. For relative quantification, the integrated optical density value defined as the sum of total pixel value-background was determined for equal-sized regions drawn around spots of interest, with background values taken below each spot of interest to account for nonspecific antibody staining in the blot. The signal of Angptl4 was normalized to the actin signal, and Student’s t-test was performed to calculate the significance of difference.

**Biochemical Studies**

**Measurement of LPL activity.** LPL activities were determined in 4-h-fasted mice as described previously (44). Adipose or kidney tissue LPL was extracted from the tissue by homogenization in 100 μl of 50 mM Tris, pH 7.4, containing 0.2% Triton X-114, 10 U/ml of heparin, and 1 mM PMSF. The extractable LPL fraction was fractionated by centrifugation at 1,500 g for 15 min at 4°C, and the supernatant was diluted with four volumes of 50 mM Tris, pH 7.4, containing 10 U/ml heparin, and 10-μl aliquots were used for LPL activity measurements in duplicate. Given the diurnal variability in the measurements of LPL activity in the mouse, mice subjected to the experimental conditions described above were euthanized in the morning and LPL enzyme activity was measured immediately after kidney tissue was harvested. LPL catalytic activity was measured as previously described using a substrate containing [3H]triolein and fetal bovine serum as a source of apoC-II (32). Extracts from adipose or kidney tissue were incubated with substrate for 1 h at 37°C. The fatty acids released during the
incubation were extracted using 3.25 ml of a mixture of methanol-chloroform-heptane 1:41:1.25:1 (vol/vol/vol) followed by 1.05 ml of 0.1 M potassium carbonate-borate buffer (pH 10.5). The methanol-water phase was separated by centrifugation at 3,000 g for 15 min at room temperature, and a 1-ml aliquot was counted using a Beckmann liquid scintillation counter. Enzyme activity was calculated and expressed as nanomoles of fatty acid released per hour per milligram protein. To measure post-heparin plasma LPL activity, mice were injected intraperitoneally (ip) with 1,000 U of heparin/kg body wt. After 15 min, the animals were euthanized and blood was collected for the isolation of plasma. LPL activity was assayed by triplicate measurements as the salt-inhibitable ability of plasma samples to hydrolyze an emulsion containing [3H]triolein as described above. To measure LPL activity in cultured adipocytes, cells were separated from the plate using a cell scraper and LPL activity was measured in 100 µl of extraction buffer as described above. Cell extracts were clarified by centrifugation at 5,000 g for 15 min. The supernatant (10 µl) was used for LPL activity measurements in duplicate as described above.

**TG measurements.** Tissues samples were homogenized using 10 volumes of a mixture of hexane/2-propanol (3/2); the suspension was filtered and evaporated to dryness. The TGs were dissolved in 100 mM Tris (pH 7.4), and total TG content was determined using a two-step TG assay kit (Sigma-Aldrich, St. Louis, MO) as described (45).

**Oil Red O Staining**

Frozen sections of kidney tissue obtained from various experimental conditions were used for oil red O staining, which was performed as previously described (40) to determine the renal accumulation of total neutral lipids.

**Immunohistochemistry of LPL and Angptl4**

Immunohistochemical staining was performed on paraffin-embedded tissue sections from mice treated with saline and cisplatin using a polyclonal anti-Angptl4 antibody obtained from Dr. S. S. Chugh, as well as a chicken polyclonal antibody against LPL provided by Dr. G. Olivecrona. We evaluated the presence of LPL and Angptl4 at 3 days after cisplatin injection in the presence of absence of the PPARα ligand WY.

**Statistical Analysis**

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

**RESULTS**

**Changes in Renal Function After Cisplatin Treatment**

Mice were fed either a regular diet or a diet containing 0.1% WY for 10 days before saline or cisplatin administration as described in METHODS. Kidney function was monitored for 3 days after intraperitoneal injection of saline or cisplatin by measuring blood urea nitrogen (BUN) and serum creatinine. Figure 1, A and B, presents the changes in BUN and creatinine seen in mice fed either a regular diet or a diet containing 0.1% WY. Comparison of the renal function between mice fed for 10 days either a regular diet or 0.1% WY-containing diet did not show differences in BUN and creatinine when treated with a single ip saline injection. Values for this control group, as shown in Fig. 1, A and B, were not significantly different on day 1 (BUN 24 mg/dl, creatinine 0.2 mg/dl) vs. day 3 (BUN 29 mg/dl, creatinine 0.3 mg/dl). Mice fed a regular diet developed acute kidney injury (AKI) on day 3. BUN increased from 26 to 132 mg/dl and creatinine increased from 0.2 to 1.3 mg/dl on day 3 after a single cisplatin injection (P < 0.001). The group of mice that received the WY diet and cisplatin did not develop significant AKI compared with mice treated with cisplatin alone (BUN increased from 23 on day 1 to 33 mg/dl on day 3, and creatinine increased from 0.2 on day 1 to 0.3 mg/dl on day 3).

**Cisplatin Causes a Reduction in Total Body Weight and Epididymal White Adipose Tissue Weight**

Wild-type mice fed a normal diet received a single ip injection of either saline solution or cisplatin. Total body weights were recorded daily for 3 days. As shown in Fig. 2, A and B, body weight, wet epididymal white adipose tissue, and epididymal white adipose tissue mass-to-body weight percentage did not change significantly in saline-treated mice. The
average epididymal white adipose tissue weight was 416 mg at day 0 and 403 mg at day 3 after saline injection. This average weight for mouse epididymal white adipose tissue in our study is consistent with previous reports by Geloen et al. (16). At 3 days after cisplatin injection, there was a 10% reduction of total body weight as shown in Fig. 2A, but also a 40% reduction of epididymal white adipose tissue mass-to-body weight percentage (down from 1.95% at day 0 to 1.17% at day 3 after cisplatin injection).
3, \( P < 0.05 \) as shown in Fig. 2B. In addition, cisplatin caused a change in the appearance of the epididymal white adipose tissue, from white (saline-treated) to red (cisplatin-treated) as shown in Fig. 2, C and D.

**Cisplatin-Treated Mice Develop Inflammatory Changes in White Adipose Tissue**

To investigate the histological changes in white epididymal adipose tissue, white epididymal adipose tissue mass was fixed in Bouin’s solution for 24 h and then processed routinely through graded alcohols and xylene for 8 h; after that, it was embedded in paraffin. Three-micrometer sections were cut, and sections were stained with hematoxylin eosin. As shown in Fig. 2, E and F, the sections of white epididymal adipose tissue obtained from saline-treated mice showed adipocytes of normal size without any inflammatory infiltrate present. Hematoxylin-eosin-stained sections obtained from cisplatin-treated mice showed focal necrosis with loss of adipocytes, and infiltration of lymphocytes and neutrophils in the necrotic area.

**Cisplatin and WY Effects on Adipose Tissue LPL mRNA Expression and Activity**

Since epididymal white adipose tissue mass was reduced by cisplatin treatment and lipoprotein lipase expressed in adipose tissue plays a critical role in the metabolism and transport of TG-containing lipoproteins, we next measured lipoprotein lipase activity in epididymal white adipose tissue isolated from saline- and cisplatin-treated mice. As shown in Fig. 3A, cisplatin inhibited LPL activity in epididymal white adipose tissues by 71% (\( P < 0.05 \)). There was no significant change in epididymal white adipose tissue LPL activity in mice fed a WY-supplemented diet before cisplatin treatment, but pretreatment with WY prevented cisplatin-mediated inhibition of LPL activity in epididymal white adipose tissue (Fig. 3A). To determine whether this inhibition of LPL activity was a direct effect of cisplatin on white adipose tissue cells, we examined the effects of cisplatin on LPL mRNA levels and LPL activity using 3T3 adipocyte cells in culture. As shown in Fig. 3, B and C, cisplatin at 12.5 and 25 \( \mu M \) also induced a dose-dependent inhibition of both LPL mRNA and LPL activity in adipocyte cells in culture.

**Cisplatin and WY Effects on Kidney Tissue LPL mRNA, Protein Levels, and Activity**

Previous studies have established the presence of kidney LPL in kidney tissue, but its regulation during AKI has not been previously studied. We examined the effects of cisplatin and the PPAR\( \alpha \) ligand WY on LPL mRNA, protein level, as well as on kidney LPL enzyme activity. As shown in Fig. 4, A–C, cisplatin caused a 50% decline in the mRNA expression of LPL in kidney tissue (Fig. 4A). Pretreatment with the PPAR\( \alpha \) ligand WY led to a minor increase in LPL mRNA (1.23-fold) but prevented cisplatin-mediated reduction of LPL mRNA levels. Similar effects were observed on LPL protein levels measured by Western blot analysis as shown in Fig. 4B. Cisplatin also inhibited LPL activity in kidney tissue by 40 \( \pm \) 10\% (\( P < 0.002 \)) in mice fed normal chow, as seen in Fig. 4C. There was no significant change in renal LPL activity in mice fed a WY-supplemented diet before cisplatin treatment, and pretreatment with WY prevented cisplatin-mediated inhibition of LPL activity in kidney tissue.

**Immunolocalization of LPL**

As shown in Fig. 4D, positive LPL staining was detected in the proximal tubules throughout the cortex in kidneys from...
saline-injected control mice. This positive staining was significantly reduced in kidney samples obtained 3 days after cisplatin injection. WY pretreatment showed a similar staining pattern to the one seen in control mice, which was not reduced after cisplatin treatment since the positive LPL staining could be detected throughout the cortex of WY+cisplatin-treated animals.

Cisplatin Increases Angptl4 mRNA Levels in Kidney, Liver, and Adipose Tissue

Our data indicate cisplatin and a PPARα ligand play an important role in regulating kidney tissue LPL expression and enzyme activity. Angptl4 has been identified recently as an important modulator of LPL activity. To further examine potential mechanisms of regulation of kidney LPL, we investigated the effects of cisplatin on mRNA levels of Angptl4 by quantitative real-time RT-PCR. Angptl4 mRNA levels in kidney tissue were increased by 9.24 ± 0.21-fold in cisplatin-treated mice compared with saline-treated mice (P < 0.001) as shown in Fig. 5A. Similarly, Angptl4 mRNA level were increased by 7.76 ± 0.63 (P < 0.001)- in liver tissue and by 1.96 ± 0.38-fold in white adipose tissue (P < 0.05) of cisplatin-treated mice.

WY Prevents Cisplatin-Mediated Increased Angptl4 Protein Levels in the Mouse Kidney

We next examined the effects of cisplatin and fibrate (WY) on protein levels of Angptl4 measured by 2D gel separation and Western blot analysis. For these studies, we used Angptl4 antibodies produced by Dr. S. Chugh’s laboratory rather than commercially available antibodies which did not give us consistent results. In our hands, Angptl4 protein was detected at very low levels in kidney tissue of saline-treated mice as several proteolytic fragments with a neutral pI and a 35-kDa molecular mass as shown by black arrows (Fig. 5). Figure 5C shows the quantification of Angptl4 protein levels normalized by actin levels demonstrating a significant increase (2.6-fold) in kidney tissue of cisplatin-treated mice. The use of the PPARα ligand WY inhibited the cisplatin-induced increase in Angptl4 protein levels. Our results suggest that PPARα ligand treatment prevents the cisplatin-induced increased expression of Angptl4 protein levels in kidney tissue.

Immunolocalization of Angptl4 Protein in Mouse Kidney Tissue

Angptl4 staining was almost completely absent in kidney tissue of saline-treated control mice. Angptl4 staining was increased in kidney tissue of cisplatin-treated mice as shown in Fig. 5D and was primarily localized to intact proximal tubules (primarily S1 and S2 segments). WY- and WY+cisplatin-treated mice also did not show significant staining for Angptl4 in the proximal tubule.

WY Reversed Cisplatin-Induced Downregulation of GPIHBP1 and Lmf1 mRNA Expression Levels

Recently, Lmf1 and GPIHBP1 have been identified as novel genes that play an important role in regulating LPL activity. We examined the effects of cisplatin and WY on renal GPIHBP1 and Lmf1 mRNA expression by quantitative real-time RT-PCR. As shown in Fig. 6, cisplatin caused a decline in the mRNA expression of GPIHBP1 and Lmf1 in kidney tissue. At day 3 after cisplatin administration, there was a 40% reduction in GPIHBP1 mRNA expression (P < 0.05) and a 60% reduction in Lmf1 mRNA expression (P < 0.01) compared with control mice. Pretreatment with WY led to a slight increase in GPIHBP1 and Lmf1 mRNA levels in wild-type mice (P > 0.05) but prevented the cisplatin-mediated reduction in kidney GPIHBP1 and Lmf1 mRNA levels.

Effects of Cisplatin and PPARα Ligand on Kidney Tissue TG Levels

Our previous studies have shown that cisplatin causes neutral lipid accumulation in the kidney cortex (39). We next examined the effect of cisplatin and WY on TG levels in kidney tissue. As shown in Fig. 7A, cisplatin-treated mice exhibited a twofold increase in TG levels in kidney tissue (P < 0.05). Pretreatment of mice with WY alone did not have a significant effect on renal TG level, but it prevented the increase in renal TG levels induced by cisplatin. We also examined the effects of cisplatin and a PPARα ligand on neutral lipid accumulation by performing intracellular stain of neutral lipids using the oil red O stain in frozen sections of kidney tissue. As shown in Fig. 7B, cisplatin treatment induced a significant accumulation of neutral lipids, which was detected as an increased intracellular red stain compared with saline-treated mice. This accumulation of neutral lipids was more pronounced than the chemical measurements of TGs given the fact that oil red O stain detects not only TGs but also nonesterified fatty acids and cholesterol accumulation after cisplatin treatment. The oil red O stain was predominantly present in the region corresponding to the proximal tubules in the kidney cortex. Pretreatment of mice with the PPARα ligand WY did not have a significant effect on neutral lipid staining but prevented the accumulation of neutral lipids induced by cisplatin.

Fig. 4. Effect of CP and WY on LPL mRNA expression (A), protein levels (B), enzyme activity (C), and immunostaining (D) in mouse kidney tissues. In mice fed regular chow, following CP treatment, LPL mRNA expression (A), protein level (B), and activity (C) were inhibited in kidney tissue. LPL mRNA, LPL protein, and LPL activity were inhibited after CP treatment in mice fed a regular chow diet. There was no significant change in LPL mRNA, protein level, and activity in mice fed a WY-supplemented diet for 10 days before CP treatment. Values are means ± SE of at least 4 independent experiments under each condition.

*P < 0.05, **P < 0.002 compared with saline-treated control mice. †P < 0.05 compared with CP-treated mice fed regular chow by unpaired Student’s t-test.

B: Western blot analysis of kidney LPL protein or β-actin. Equal quantities of protein from 4 different conditions, i.e., control, CP, WY, and WY+CP, were loaded and separated in the same gel and then subjected to Western blot analysis as described. D: Immunostaining for kidney LPL. Positive LPL staining can be seen in the proximal tubules throughout the cortex in kidneys from control mice, which is significantly reduced after CP treatment. The staining in WY-pretreated animals is similar to control and it does not change after CP administration in the WY+CP-treated kidneys. WY pretreatment also protected kidney morphology, since necrotic tubules can be seen only in CP-treated mice (*) Arrows show that LPL immunostaining is present in proximal tubules.
DISCUSSION

The current studies expand on previous observations and address potential mechanism(s) by which cisplatin induces the accumulation of TG and neutral lipids in kidney tissue (40). In our new studies, we found that cisplatin directly mediates white adipose tissue breakdown by significantly reducing the white epididymal fat pad mass which is accompanied by increased inflammation and cell death as shown in Figs. 2 and
3. The mechanisms responsible for these systemic effects of cisplatin on white adipose tissue are not entirely clear but seem to be specific for this nephrotoxin. Ischemia for 45 min followed by 24 h of reperfusion injury did not cause significant changes in the weight of the white epididymal fat mass (results not shown). White adipose tissue stores fat as a source of energy in the case of fluctuations in food availability, but also serves as an endocrine organ secreting leptin and other hormones that signal energy status to the brain and regulate appetite and insulin sensitivity (2, 15, 48). We speculate that reduced epididymal white fat pad mass is part of a systemic inflammatory response induced by cisplatin. In fact, previous studies have reported that cisplatin-mediated nephrotoxicity is accompanied by activation of proinflammatory cytokines and chemokines (27, 43). Ramesh et al. (43) found that cisplatin injection increases the levels of TNF-α in the serum, kidney tissue, and urine and that the inhibition of TNF-α, using TNF-α-deficient mice or TNF-α inhibitors, ameliorated cisplatin-induced renal dysfunction. TNF-α in turn has been shown to stimulate lipolysis in white adipose tissue and could represent one of the factors involved in reduced adipose tissue mass (23, 56). A recent study investigated the direct effects of uremic serum on human adipocyte function (4). Using this in vitro system, the authors measured released glycerol as well as mRNA levels of the lipid-associated protein perilipin (PLIN). Axelsson et al. (4) concluded that undefined circulating factors in chronic kidney disease (CKD) patients increase basal lipolysis in human adipocytes in vitro, probably by attenuating the expression of the lipolytic regulator PLIN. Therefore, the presence of white adipose tissue lipolysis mediated by increased serum levels of TNF-α and the presence of circulating factors caused by uremia during AKI could explain the effects of cisplatin on reducing white adipose tissue mass. Our studies also show that, in addition to reducing epididymal white adipose tissue mass, cisplatin reduces epididymal white adipose tissue LPL activity. Although we did not explore in detail the mechanisms involved in the inhibition of LPL activity in epididymal white adipose tissue, our data suggest that both reduced epididymal white adipose tissue mass as well as increased expression of Angptl4 could contribute to the observed inhibition of adipose LPL activity.

Next, we investigated the effects of cisplatin and the PPARα ligand WY on expression and enzyme activity of kidney tissue LPL and Angptl4, a protein that appears to play a major role in LPL function. LPL is the enzyme responsible for the hydrolysis of core TGs in chylomicrons, producing chylomicron remnants and intermediate-density lipoproteins (14, 18, 54). This enzyme is primarily expressed in tissues that use fatty acids for fuel or store large amounts of TGs, such as parenchymal cells of the adipose tissue, skeletal muscle, heart, and kidney cortex, and is secreted to the endothelium of local blood vessels (53). Previous studies by Olivecrona’s group (46) demonstrated that LPL is present in mouse kidney proximal tubular epithelial cells; however, the regulation of LPL enzyme activity or its expression during acute kidney injury has not been previously examined. We found that similar to the effects of reducing epididymal white adipose tissue mass and LPL activity, cisplatin also inhibited the expression and enzyme activity of kidney LPL. Our Western blot analysis demonstrated reduced expression of LPL protein in kidney tissue of cisplatin-treated mice, and immunohistochemical studies further identified reduced proximal tubule LPL protein after cisplatin treatment. We also found that cisplatin-mediated inhibition of kidney tissue LPL activity was accompanied by increased expression of Angptl4 mRNA and protein levels. In addition, we show by immunohistochemical studies that increased expression of Angptl4 protein by cisplatin occurs predominantly in proximal tubules. Our studies are the first to show a correlation between inhibition of kidney tissue LPL activity and increased expression of Angptl4 protein in this model of cisplatin-mediated AKI. This would be logical because the tubular epithelial cells consume much energy and derive it mainly from fatty acids. Experiences with other tissues indicate that LPL is usually produced by the cells that will take up and metabolize fatty acids. Our studies in the cisplatin model as well as in the ischemia-reperfusion injury model document inhibition of kidney tissue LPL activity during AKI. This is in contrast to what has been described in the 5/6 nephrectomy model of CKD, where Vaziri et al. (50–53) found a significant reduction of gene expression, protein abundance, and enzyme activity of LPL in non-kidney tissues including adipose tissue, skeletal muscle, and myocardium. Those findings in the animal model of CKD could also explain why patients with CKD develop hypertriglyceridemia, impaired clearance of VLDL and chylomicrons, TG enrichment of LDL, and HDL (19, 51).

Our studies show that cisplatin-mediated inhibition of LPL expression and enzyme activity correlates with increased expression of Angptl4 and increased accumulation of intracellular TG in kidney tissue and, more specifically, in the proximal tubule. Since reduced kidney LPL activity by cisplatin is expected to reduce the amount and TG and fatty acids delivered to kidney tissue, we propose that despite inhibiting renal LPL by upregulation of Angptl4 protein, cisplatin treatment raises TG levels in kidney tissue by impairing fatty acid oxidation, which can be prevented by administration of a PPARα agonist. Indeed, our previous studies have demonstrated that ischemia-reperfusion and cisplatin-mediated AKI inhibit fatty acid oxidation in kidney tissue, leading to the accumulation of neutral lipids including nonesterified fatty acids and TGs. We also showed in those studies that the use of

Fig. 5. Effect of CP and WY on angiotensin protein-like 4 (Angptl4) mRNA expression (A), protein levels (B), activity (C), and immunohistochemical localization of Angptl4 (D) in mouse kidney tissues. A: effects of CP on mouse Angptl4 mRNA levels in the kidney, liver, and epididymal white adipose tissues. B: representative autoradiograph of 2-dimensional gel electrophoresis and Western blot analysis from mouse kidney tissue homogenates. The arrows point toward a 35-kDa neutral pl Angptl4 protein in control, CP-, WY-, and WY+CP-treated mice. C: quantitative densitometry of the 35-kDa neutral pl Angptl4 protein shown in B after normalization with β-actin. Values are means ± SE of mRNA levels. Data were obtained from at least 4 independent experiments under each condition. *p < 0.05, **p < 0.001 compared with saline-treated WT mice. 1P < 0.05 compared with CP-treated mice in unpaired Student’s t-test. D: immunostaining for Angptl4 in kidney tissue. Angptl4 staining is mostly absent in control kidney tissue, and the arrows show that Angptl4 immunostaining is significantly increased in kidney tissue of CP-treated mice, and it is primarily localized to intact proximal tubules (primarily S1 and S2 segments). WY- and WY+CP-treated mice do not show significant staining for Angptl4 in the proximal tubule. Necrotic tubules can be seen only in CP-treated mice (*).
needed to demonstrate a cause-effect relationship in kidney tissue between increased Angptl4 expression and reduced kidney LPL activity. In a recent study by Clement et al. (12), the authors injected rats with nephrotoxic serum and demonstrated increased expression of Angptl4 in podocytes. In addition, these authors showed that injection of a single dose of puromycin aminonucleoside or use of the model of passive Heymann nephritis led to a significant increase in the expression of Angptl4 protein in podocytes. To further examine the consequences of increased Angptl4 expression in podocytes, the authors developed podocyte-specific Angptl4 transgenic rats, as well as adipose tissue-specific Angptl4 transgenic rats. Podocyte-specific transgenic overexpression of Angptl4 in rats induced nephrotic range proteinuria, loss of glomerular basement mem-

a PPARα ligand or proximal tubule PPARα transgenic mice ameliorates AKI by reducing proximal tubule cell injury and lipid accumulation (26, 28, 29).

A variety of proteins that regulate LPL activity have been identified including apo CII, apo CIII, apo A5, and angiopoietin-like proteins 3 and 4 (7, 8, 17, 21, 30, 47). Angptl4 has been shown to convert catalytically active LPL to inactive monomers (47). In recent years the angiopoietin-like proteins Angptl3 and Angptl4 have emerged as novel modulators of LPL activity. Studies in transgenic animals supported by in vitro experiments have demonstrated that Angptl3 and Angptl4 impair TG clearance by inhibiting LPL activity. In humans, genetic variation within the Angptl3 and Angptl4 genes contributes to variation in plasma TG and HDL levels, thereby validating the importance of angiopoietin-like proteins in the regulation of lipoprotein metabolism in humans (30). Although our study shows that increased expression of Angptl4 in the proximal tubule is associated with reduced kidney tissue LPL activity during cisplatin-mediated AKI, additional studies are

Fig. 6. Effect of CP and WY on renal glycosylphosphatidylinositol-anchored-HDL-binding protein (GPIHBP1; A) and lipase maturation factor 1 (Lmf1; B) mRNA levels in mouse kidney tissue. Real-time RT-PCR was performed using total RNA isolated from kidney tissue of mice fed a normal chow (group saline and CP) or WY-containing chow (groups WY and WY+CP). Tissues were collected at day 3 after either saline (group saline and WY) or CP (groups CP and WY+CP) IP injection. Values are means ± SE of mRNA levels. Data were obtained from at least 4 independent experiments under each condition.

Fig. 7. Effect of CP and WY on triglyceride content (A) and neutral lipid accumulation (B) in mouse kidney tissue. Mice were fed a regular chow or a WY-supplemented diet for 10 days before they were treated with CP as described in METHODS. Triglyceride content is expressed as milligrams triglyceride per milligram tissue protein (A). B: frozen sections were obtained from animals subjected to 4 experimental conditions, processed, and stained with oil red O dye as described in METHODS. Depicted are pictures obtained from oil red O staining of kidney sections from control, CP-, WY-, and WY+CP-treated mice. Data were obtained from at least 4 independent experiments under each condition.

*P < 0.05, **P < 0.01 compared with saline-treated WT mice. †P < 0.05 compared with CP-treated WT mice by unpaired Student’s t-test.

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brane charge, and foot process effacement, whereas transgenic expression specifically in the adipose tissue resulted in increased circulating Angptl4 but not proteinuria. In addition, these authors noted that Angptl4 secreted from podocytes in some forms of nephrotic syndrome lacked normal sialylation, and that the use of sialic acid precursor N-acetyl-
mannosamine in podocyte-specific Angptl4 transgenic rats increased Angptl4 sialylation and reduced albuminuria (12). Altogether, these results suggest that Angptl4 plays a key role in the pathogenesis of nephrotic syndrome. Although we did not measure Angptl4 protein expression in the serum of cisplatin-treated mice, the observed systemic response of mice receiving cisplatin with increased expression of Angptl4 in adipose tissue, liver, and kidney tissue again suggests the presence of a generalized metabolic response in various organ systems after cisplatin injection. Additional studies using proximal tubule Angptl4 transgenic mice as well as Angptl4 –/– mice are necessary to further understand the function of increased Angptl4 expression in the proximal tubule and its effect on inhibiting kidney LPL activity after cisplatin injury.

Finally, we examined the effects of cisplatin injury on the expression of kidney GPHBP1 and Lmf1, two important modulators of LPL activity. The function of GPHBP1 in lipolysis was clarified when it was shown that GPHBP1 knockout mice on a chow diet have milky plasma with high plasma TG levels (6). By immunohistochemistry, GPHBP1 is located in capillary endothelial cells, and GPHBP1-expressing cells bind LPL avidly. Furthermore, Davies et al. (13) showed that GPHBP1 shuttles LPL from the interstitial spaces of tissues where it is secreted by parenchymal cells into the capillary lumen, the site where it needs to be to hydrolyze TGs in lipoproteins. A recent study using 124I-labeled GPHBP1-specific monoclonal antibodies, along with PET scanning, yielded new and unexpected insights into GPHBP1 expression and function (34). Significant amounts of GPHBP1 were found in the lungs, liver, and kidney, tissues that are not thought to have important roles in LPL-mediated processing of TG-rich lipoproteins. Lmf1 has been identified as the gene responsible for the combined lipase deficiency in mice with severe hypertriglyceridemia (35, 37). This chaperone is involved in the posttranslational maturation of lipase polypeptide chains within the endoplasmic reticulum, allowing the assembly of lipase monomers into active homodimers or in the stabilization of dimers already formed. We find that GPHBP1 and Lmf1 mRNA levels are both reduced by cisplatin injury. Use of a PPARα ligand restored the expression of GPHBP1 and Lmf1 in kidney tissue back to normal levels.

In summary, our studies demonstrate reduced lipolytic processing of kidney tissue LPL after cisplatin injury. We also present evidence for increased expression of proximal tubule Angptl4 and reduced expression of GPHBP1 and Lmf1 as potential cellular mechanisms responsible for cisplatin-mediated inhibition of kidney tissue LPL activity. Additional studies, including in situ hybridization and immunolocalization studies as well as functional studies of GPHBP1 and Lmf1 activities in kidney tissue, are needed to further understand the role of reduced LPL activity in kidney tissue during AKI.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.L., K.N., G.R., S.M.A., B.S., S.A., S.K., and D.P. performed experiments; S.L., K.N., G.R., N.A., J.M., and D.P. prepared figures; S.L. and D.P. drafted manuscript; S.L. and D.P. approved final version of manuscript; N.G., J.M., G.O., S.S.C., S.K., and D.P. interpreted results of experiments; D.P. provided conception and design of research; D.P. analyzed data; D.P. edited and revised manuscript.

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