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# Suramin protects from cisplatin-induced acute kidney injury.

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<https://doi.org/10.18297/etd/2217>

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SURAMIN PROTECTS FROM CISPLATIN-INDUCED ACUTE KIDNEY INJURY

By

Tess Dupre

A Thesis  
Submitted to the Faculty of the  
School of Medicine of the University of Louisville  
in Partial Fulfillment of the Requirements  
for the Degree of

Master of Science in Pharmacology and Toxicology

Department of Pharmacology and Toxicology  
University of Louisville  
Louisville, KY

August 2015



SURAMIN PROTECTS FROM CISPLATIN-INDUCED ACUTE KIDNEY INJURY

By

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B.S., University of Louisiana at Monroe, 2012

Thesis Approved on

July 1, 2015

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## ACKNOWLEDGEMENTS

I would like to acknowledge my mentor, Dr. Leah J. Siskind, I am grateful for her guidance and support. Her confidence in my abilities has been instrumental in the success of my research. Many thanks to my committee, Dr. Rick Schnellmann, Dr. Levi Beverly, Dr. Chris States, and Dr. Gavin Arteel for their comments and assistance.

## ABSTRACT

### SURAMIN PROTECTS FROM CISPLATIN-INDUCED ACUTE KIDNEY INJURY

Tess V. Dupre

July 1, 2015

In rodent models, suramin improves recovery following acute kidney injury (AKI). We hypothesized that suramin would be useful in protection against cisplatin-induced AKI. This hypothesis was tested by pre-treating C57BL/6j mice with suramin prior to cisplatin. Our data indicates that suramin protects the kidney from injury by decreasing cisplatin-induced decreases in kidney function. Renal histology indicated that suramin significantly protected from cisplatin-induced AKI. Data indicate that suramin pretreatment attenuated mRNA expression of pro-inflammatory cytokines and chemokines following cisplatin treatment, while also decreasing markers of cell stress and cell death. We utilized the same experimental design with 10-month old FVB mice expressing mutant Kras driven lung adenocarcinomas. Assessment of lung histology and kidney function indicated that suramin protected mice from cisplatin-induced AKI and did not inhibit cisplatin's anti-tumor efficacy. These results suggest that suramin shows great potential as a renoprotective agent for the treatment/ prevention of cisplatin-induced AKI.

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## INTRODUCTION

The kidneys are responsible for many essential physiological functions, including the following: filtration and elimination of metabolic and toxic wastes from the blood; regulation of the internal fluid environment to maintain proper fluid volume and tonicity, pH balance, and electrolyte composition; essential endocrine functions such as erythropoiesis and blood pressure regulation; and the metabolism and excretion of many drugs. A rapid loss in kidney function, commonly referred to as acute kidney injury (AKI), can result in a myriad of clinical manifestations and sequelae (1). Unfortunately, there is currently no single definition of AKI, but rather multiple slightly different and widely used definitions of AKI. While all of the accepted clinical definitions of AKI monitor changes in serum creatinine and urine output to diagnose and classify the severity of clinical presentations of AKI, the actual diagnosis and prognosis of each AKI case will differ slightly amongst clinics depending on the definition utilized (1). The incidence of AKI is more than 5,000 cases per million per year for non-dialysis-requiring patients, and the incidence of AKI for dialysis-requiring patients is greater than 295 cases per million per year (1, 2). AKI complications are observed in 1-9% of hospital inpatients, with 40% of these patients being admitted to the intensive care unit due to AKI complications; more than 60% of patients in the intensive care unit have some sort of AKI episode during their stay, having 50-70% increased mortality rate (1, 3, 4). AKI can result from a number of causes including pre-renal (loss of blood flow to the kidney), post-renal (urinary tract obstruction), or intrinsic AKI (4). Intrinsic AKI is the most common form of AKI (4) and can be divided into three subcategories: acute glomerular nephritis, acute interstitial nephritis, and acute tubular necrosis (4). A common cause of intrinsic kidney injury,

specifically acute tubular necrosis, is nephrotoxic pharmaceutical agents, which account for 55-60% of hospital inpatient AKI cases (4). There are a number of pharmaceutical agents including many antibiotics and anti-cancer chemotherapeutics that have a severe side effect of nephrotoxicity. Cisplatin (cis-diamminedichloridoplatinum(II)) is a commonly used chemotherapeutic in the treatment of many solid tumor cancers such as head and neck, bladder, ovarian, lung, and testicular cancers (5-7). Cisplatin is a simple, small, inorganic platinum-based drug (5-7). It induces cell death by binding DNA, leading to the formation of intra- and inter-strand crosslinks (6, 7); this can result in the formation of faulty DNA templates, leading to cell cycle arrest and ultimately cell death. Cancer cells are particularly susceptible to the cytotoxic mechanism of action of cisplatin largely because they are rapidly dividing, which increases the likelihood of cisplatin binding to DNA and eliciting its cytotoxic action. Cisplatin is eliminated through renal excretion, where it is metabolized to a nephrotoxic metabolite and consequently has a dose-limiting side effect of nephrotoxicity (5, 8-10) observed in over 30% of patients administered cisplatin (10).

Cisplatin, once metabolized to its nephrotoxic metabolite, induces a myriad of physiological responses in the kidney resulting in kidney injury and ultimately kidney failure (5, 9-12). While some of the processes involved in cisplatin-induced nephrotoxicity are understood, many are not well elucidated and there are no renoprotective strategies for protecting the kidney from cisplatin (nor any other nephrotoxic agent). For this reason, patients suffering from cisplatin-induced kidney injury must be removed from cisplatin treatment or placed on a lower dose, resulting in the ineffective treatment of their cancers. Thus, there is still a clear need to gain a better understanding of the mechanisms by which cisplatin causes nephrotoxicity so that novel targets can be identified for the development of renoprotective strategies. This would

widen the therapeutic window of cisplatin in cancer treatment, making it more effective at reducing tumor burden.

Cisplatin is accumulated in the kidney at concentrations five-times higher than those observed in serum (9). In the kidney, cisplatin is concentrated primarily within the proximal tubules of the nephron (5, 8-10). Cisplatin enters the proximal tubular portion of the nephron in a number of ways. One way cisplatin enters the proximal tubule is via glomerular filtration in part because of cisplatin's small size (9, 10). Cisplatin is also a known substrate for at least two transporters, the organic cation transporter-2 (OCT2) and copper transporter-1 (CTR1), which are both expressed basolaterally on proximal tubule cells (5, 9, 12, 13). OCT2 expression is higher in the kidney than any other tissue in the body (14). It has been shown both *in vivo* and *in vitro* that cisplatin is a substrate for OCT2; importantly, inhibition of OCT2 with cimetidine or knockdown of OCT2 protects against cisplatin-induced injury both *in vitro* and *in vivo* (14-16). Once cisplatin enters the proximal tubule cells through these transporters it is transported into the lumen of the proximal tubule where it is metabolized to a nephrotoxic metabolite. The transporter responsible for the efflux of cisplatin out of proximal tubule cells into the lumen is the multidrug extrusion transporter-1 (MATE-1), which is found on the apical surface of proximal tubule cells (17).

The metabolism of cisplatin to a nephrotoxic metabolite is a multistep process that first begins with the conjugation of cisplatin to glutathione (5, 8-10). LLC-PK<sub>1</sub> cells treated with the cisplatin-glutathione conjugates have significantly less viability than unconjugated cisplatin treated cells (8). Thus, cisplatin-glutathione conjugates are more nephrotoxic than unconjugated cisplatin, suggesting that cisplatin is in fact metabolized to a more nephrotoxic chemical species. Cisplatin-glutathione conjugates are substrates for gamma-glutamyl transpeptidase (GGT) (8), an enzyme expressed on the apical surface of proximal tubule cells. GGT is responsible for the metabolism of cisplatin-

glutathione conjugates to form a cisplatin-cysteinyglycine-conjugate, a substrate for aminodipeptidase (8, 18). Aminodipeptidase is another enzyme highly expressed on the apical surface of proximal tubule cells, and is responsible for the cleavage of the cisplatin-cysteinyglycine-conjugate to a cisplatin-cysteinyconjugate. The metabolites formed following aminodipeptidase reactions are substrates for cysteine-conjugate  $\beta$ -lyase (19), an enzyme found intracellularly in renal proximal tubular cells. Thus, cysteinyconjugates are metabolized by cysteine-conjugate  $\beta$ -lyase to form a reactive thiol conjugate (13, 20, 21). Inhibition of either GGT or cysteine-conjugate  $\beta$ -lyase resulted in protection from cisplatin induced cell death *in vitro*, indicating that the reactive thiol conjugate formed after the cysteine-conjugate  $\beta$ -lyase catalyzed reaction is the final nephrotoxic metabolite of cisplatin (8, 22).

Due to the complexity of cisplatin's nephrotoxic mechanism of action, the development of renoprotective agents remains a challenge. In the kidney, cisplatin is metabolized to its nephrotoxic metabolite, which induces kidney injury and ultimately kidney failure (5, 9-12). Cisplatin-induced nephrotoxicity involves several biological responses, including mitochondrial dysfunction, activation of cell death pathways, generation of reactive oxygen species, and the mounting of a robust inflammatory response (5, 10). At lower concentrations, cisplatin induces apoptosis, whereas at higher concentrations it induces necrosis (9, 11, 23). Both mitochondrial dysfunction and cell death have been shown to be up and downstream of the induction of inflammatory pathways, which act to further perpetuate cisplatin-induced kidney injury (24-26).

The inflammatory response associated with cisplatin-induced AKI is known to have many components such as production of inflammatory cytokines and chemokines, activation of resident macrophages, and infiltration of immune cells into the kidney (10, 12). Many pro-inflammatory cytokines and chemokines have been implicated in cisplatin-

induced AKI, such as tumor necrosis factor-  $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and monocyte chemotactic protein (MCP-1) (10, 12). TNF- $\alpha$  has been shown to be a major mediator of kidney injury following cisplatin treatment. Inhibition of TNF- $\alpha$  synthesis or use of a neutralizing TNF- $\alpha$  antibody attenuates cisplatin-induced AKI in rodents (26, 27). Mice deficient in TNF- $\alpha$  as well as TNF- $\alpha$  receptor-1 deficient mice are protected from cisplatin-induced kidney injury (26, 27). While a role for TNF- $\alpha$  is well established, the role of other cytokines and chemokines in cisplatin-induced AKI is not well established. Cytokine production (IL-1 $\beta$ , IL-18, and IL-6) following cisplatin treatment is in part mediated via caspase 1 (28). While caspase 1-knockout mice have decreased production of IL-1 $\beta$ , IL-18, or IL-6 following cisplatin treatment and are protected from cisplatin-induced AKI. Specific inhibition IL-1 $\beta$ , IL-18, or IL-6 was insufficient to protect mice from cisplatin-induced AKI (28). The chemokine (C-X-C motif) ligand 1 (CXCL1) has also been implicated in the pathogenesis of cisplatin-induced AKI as CXCL1 receptor-deficient mice are protected from cisplatin-induced AKI (29). Many of these recent studies, among many others in the literature, only inhibit one arm of the inflammatory response; however an agent that can antagonize multiple arms of the inflammatory response may be an invaluable renoprotective approach for cisplatin-induced AKI.

Interestingly, purinergic type 2 (P2) receptors have been implicated in many arms of the inflammatory response. Both dying cells and damaged tissue release ATP as danger signals, which elicit an inflammatory response (30). P2 receptors recognize danger signals including ATP and play a crucial role in inducing activation of the inflammatory response (30). P2 receptors are known to be expressed on multiple inflammatory cell types such as mast cells, T cells, macrophages, and neutrophils (30), which have all been shown to infiltrate the kidney during cisplatin-induced AKI (10, 12). More specifically, P2 receptors in macrophages play a role in macrophage spreading

and chemotaxis (30) and regulate the activation of caspase-1, leading to the production of the pro-inflammatory cytokines IL-18 and IL-1 $\beta$  (30, 31). P2 receptors are expressed not only on inflammatory cells, but also in every tissue in the body. There are many P2 receptor subtypes that are expressed in renal proximal tubule cells, the main site of cisplatin-induced injury in the kidney, and/or the inflammatory cell types involved in the inflammatory response associated with cisplatin. For example, P2Y6 is expressed in renal proximal tubule cells and is known to be involved in the release of many chemokines and cytokines (30, 32). This receptor along with other P2 receptor subtypes such as P2Y2, P2Y12, and P2X7 have been implicated in the recruitment of monocytes, macrophages, and neutrophils (30, 32), all of which infiltrate the kidney following treatment with cisplatin (12). Since P2 receptors play a regulatory role in chemotaxis and the production of pro-inflammatory cytokines and are located in proximal tubule cells, their inhibition may prove to be protective from cisplatin-induced AKI.

Suramin, a pan P2 receptor antagonist, is a polysulfonated naphthylurea compound originally designed for the treatment of trypanosomiasis. Eichhorst *et al.* have previously demonstrated that pretreatment or co-administration of suramin with fulminant protects mice from liver damage via inhibition of death receptor-induced apoptosis (33). Suramin has also been shown to speed recovery in rodent models of kidney diseases (34-40). In diabetic nephropathy, unilateral ureteral obstruction, and the remnant kidney, post-treatment of suramin was shown to dampen the inflammatory response and reverse the associated fibrotic phenotypes (34-40). Also, in ischemia/ reperfusion and glycerol-induced AKI, suramin post-treatment improves recovery by dampening the inflammatory response and promoting renal proximal tubule cell proliferation (35). A role for the P2 receptors in cisplatin-induced AKI has not been examined and suramin has never been tested in models of cisplatin-induced AKI. Likewise, it is unknown if prophylactically blocking the P2 receptors with suramin will reduce kidney injury. We hypothesized that

inhibition of the P2 receptors would attenuate the inflammatory response upon cisplatin treatment and protect the kidney from injury.

To test this hypothesis, we inhibited the P2 receptors via suramin and then treated mice with cisplatin. Renal function, pathology, inflammation, and markers of cell death were assessed. Our data presented below indicate that pretreatment with suramin protects the kidney from cisplatin as evidenced by improved markers of renal function, pathology, reduced expression of inflammatory cytokines and chemokines, and cell death.

## MATERIAL AND METHODS

### **Reagents and Antibodies**

The following antibodies were purchased from Cell Signaling: inositol requiring enzyme 1a (IRE1 $\alpha$ ; Cat. No. 3294), Cleaved Caspase 3 (Cat. No. 9664), Cleaved Caspase 8 (Cat. No. 9252), C/EBP-homologous protein (CHOP; Cat. No. 2895), c-Jun N-terminal kinases (JNK; Cat. No. 9258), p-JNK (Cat. No. 4668), proliferating cell nuclear antigen (PCNA; Cat. No. 13100), and phosphorylated histone H2Ax (Cat. No. 9718). and  $\beta$ -actin were purchased from Santa Cruz (Dallas, TX). Both Suramin (S2671) and Cisplatin (P4394) were purchased from Sigma Aldrich (St. Louis, MO)

### **Animals**

C57BL/6j mice were purchased from The Jackson Laboratory (Bar Harbor, ME) at 8 weeks of age and experimentation was started at 9 weeks. All mice were maintained on a 12-hour light/ 12-hour dark cycle and provided food and water *ad libitum*. Animals were maintained under standard laboratory conditions. All animal procedures were approved by the Institutional Animal Care and Use Committee and followed the guidelines of the American Veterinary Medical Association. Suramin (1 or 10 mg/ kg) in PBS (200  $\mu$ L / animal) was administered by tail vein injection 72 h prior to cisplatin. Cisplatin at 20 mg/ kg in PBS (200  $\mu$ L / animal) was administered by i.p. injection. Forty-eight hours after cisplatin injection, mice were put into metabolic cages for urine collection for 24 h and then euthanized. Blood was drawn and serum prepared and stored at -80°C. The kidneys were flash frozen in liquid nitrogen or fixed in 10 % neutral buffered formalin. Eight to ten month old FVB mice expressing mutant driven lung tumors were obtained as



a gift from Dr. Levi Beverly. These bi-transgenic mice were made by crossing CCSP-rtTa activator mice to Tet-op-K-ras<sup>G12D</sup>. Genotyping was performed via PCR as described previously (41).

### **BUN and Serum Creatinine**

Blood Urea Nitrogen (BUN, DIUR-500) and Serum Creatinine (C7548-120), were determined using kits from Bioassay Systems (Hayward, CA) and Point Scientific Inc. (Canton, MI), respectively following the manufactures' instructions.

### **Protein Quantification, Western Analysis, and ELISAs**

Homogenates were made from kidney cortex by homogenization in 20 mM HEPES buffer containing a Complete Protease Inhibitor Cocktail Tablet and Phosphatase Inhibitor Cocktail Tablets (Roche, Indianapolis, IN). Homogenates were centrifuged at 15,000 X g for 10 min at 4° C. Supernatants were removed, mixed, aliquotted, and stored at -80°C until use. Protein concentrations were determined using Bradford Reagent (Bio-Rad, Hercules CA). Kidney homogenate (40 µg) was separated on 4–12% gradient Tris-Glycine-SDS polyacrylamide gels and transferred to PVDF membranes that were blocked in 5% (w/v) dried milk in tris buffered saline containing 0.1% (v/v) Tween 20 (TBST) for 1 h. Membranes were incubated with 1:1000 dilutions of primary antibody overnight at 4°C. The next morning, membranes were washed 3 times for 5 min each with TBST containing 5 % (w/v) dried milk. After incubation for 2 h at room temperature with secondary antibodies conjugated with horseradish peroxidase (1:10,000, in TBST containing 1.25% (w/v) dried milk), membrane proteins were detected by chemiluminiscence substrate. ELISAs for kidney injury molecule-1 (KIM-1) (DT1817, R&D systems, Minneapolis, MN) and neutrophil gelatinase associated lipocalin (NGAL) (DY1857 R&D Systems) were performed on the urine as directed by the manufacturer.

### **Gene Expression**

Total RNA was isolated using RNA-STAT 60 (TEL-TEST Inc., Friendswood, TX) using mini-Bead-beater glass beads and mini Bead Beater machine (Cole-Palmer, Vernon Hills, IL). Other than using the bead beater glass bead to disrupt the tissue, we used the protocol provided by RNA-STAT 60 manufacturer. cDNA was made from 1 µg of total RNA using iScript cDNA synthesis Kit (Bio-Rad) following manufacturers' instructions. Gene specific cDNAs were quantitated using real-time PCR using either Sybr green or predesigned TaqMan assays. Primers used in real-time PCR reaction quantitated by Sybr green were designed such that each primer set bound to exons separated by an intron of at least 600 bp to minimize the likelihood of genomic DNA amplification. Melt curve analysis was also performed to ensure only one PCR product was being amplified. Transcripts quantitated using Sybr green included TNF $\alpha$  (5'-aatggcctccctctcatcagtt-3' and 5'-ccacttggtggttgctacga-3') and the housekeeping gene for normalization B2M (5'-ttctggtgctgtctcactga-3' and 5'-cagtatgttcggctccattc-3') using Sso Fast Evagreen Supermix (Bio-Rad) following manufacturer's instruction. Chemokine (C-X-C) ligand-1 (Cxcl1; Mm04207460\_m1), Chemokine (C-C) ligand-2 (Ccl2 or Mcp1; Mm00441242\_m1), Interleukin-1 $\beta$  (Il1 $\beta$ ; Mm0043228\_m1), Interleukin-6 (IL6; Mm00446190\_m1) were purchased from Life Technologies (Grand Island NY) and used in combination with 2x Gene expression Master Mix (Life Technologies). Primers for quantitation of hepatitis A cellular receptor-1 (Havcr1 or Kim-1) were purchased from Bio-Rad and used in combination with Sso Advance Universal Probe Master Mix (Bio-Rad) following manufacturer's instruction.

## **Histology**

Kidney sections (5 µm) were stained with hematoxylin and eosin and periodic acid schiff (PAS), and the degree of morphologic changes was determined by light microscopy in a blinded fashion. The following measures were chosen as an indication of morphologic

damage to the kidney after treatment with vehicle, suramin 10 mg/ kg, cisplatin 20 mg/ kg, or cisplatin and suramin 10 mg/ kg: proximal tubule dilation, brush-border damage, proteinaceous casts, interstitial widening, and necrosis. These measures were evaluated on a scale from 0 to 4, which ranged from not present (0), mild (1), moderate (2), severe (3), and very severe (4). Lung sections (5  $\mu$ m) from animals 48 h after cisplatin administration were stained with hematoxylin and eosin and PAS.

### **Immunohistochemistry**

Lung sections were rehydrated in xylene followed by an ethanol gradient. Antigen retrieval was performed in citric acid buffer pH 6.0 at 95 °C in a steamer for 30 min. Endogenous peroxidases were inhibited with 3% hydrogen peroxide for 10 min, followed by two 5-minute PBS washes. Slides were blocked with 5% normal goat serum in 0.1% TBST for 1 h at room temperature. Anti- phosphorylated H2A.x (aka  $\gamma$ H2A.x) (1:480) in 5% normal goat serum containing TBST was added to sections and incubated overnight at 4 °C. The following morning, slides were washed three times with PBS for 5 minutes each. Biotinylated goat anti-rabbit IgG antibody (1:200) (Vector laboratories, BA-1000) was added to each section and incubated for 30 minutes at room temperature. Slides were then rinsed twice with PBS (5 minutes each). Vector ABC reagent (Vector laboratories, PK-7100) was added to each section and incubated for 30 minutes at room temperature. Slides were rinsed twice with PBS, followed by the addition horse-radish peroxidase (HRP), which was detected with NovaRed Substrate (Vector Laboratories, SK-4800; added to each section for 10 minutes). Slides were rinsed in tap water for 5 minutes, counterstained with modified mayer's hematoxylin (thermo scientific, 72804), followed by dehydration in an ethanol gradient to xylene and mounting with Permount (Fisher Scientific, SP15).

### **Cell Viability**

A549 cells were maintained in Dulbecco's modified Eagle's medium, high glucose, and 5% (v/v) fetal bovine serum (FBS). A549 cells seeded in 96-well dishes (5,000 cells per well) were treated with either cisplatin (P4394, Sigma-Aldrich, St. Louis, MO) alone or cisplatin plus 50  $\mu$ M suramin (S2671, Sigma-Aldrich) 48 h after plating in a total volume of 200  $\mu$ l per well. All treatments were done in triplicate. Cells were incubated for 24 h and 10% (v/v) (20  $\mu$ l) Alamar Blue reagent (DAL1100) (Invitrogen, Grand Island, NY) was added. Plates were then incubated for 4 h and the fluorescence of Alamar Blue reduction determined on a BioTek HT Synergy plate reader (540 nm excitation, 594 nm emission). Wells containing only complete DMEM media and vehicle plus 20  $\mu$ l of Alamar Blue were averaged and subtracted from all experimental readings. Drug treatment regimens were then normalized to either vehicle treated cells or to wells containing only 50  $\mu$ M Suramin. Each graph shown is a representative experiment of at least three biological replicates, with error bars representing standard error of the mean.

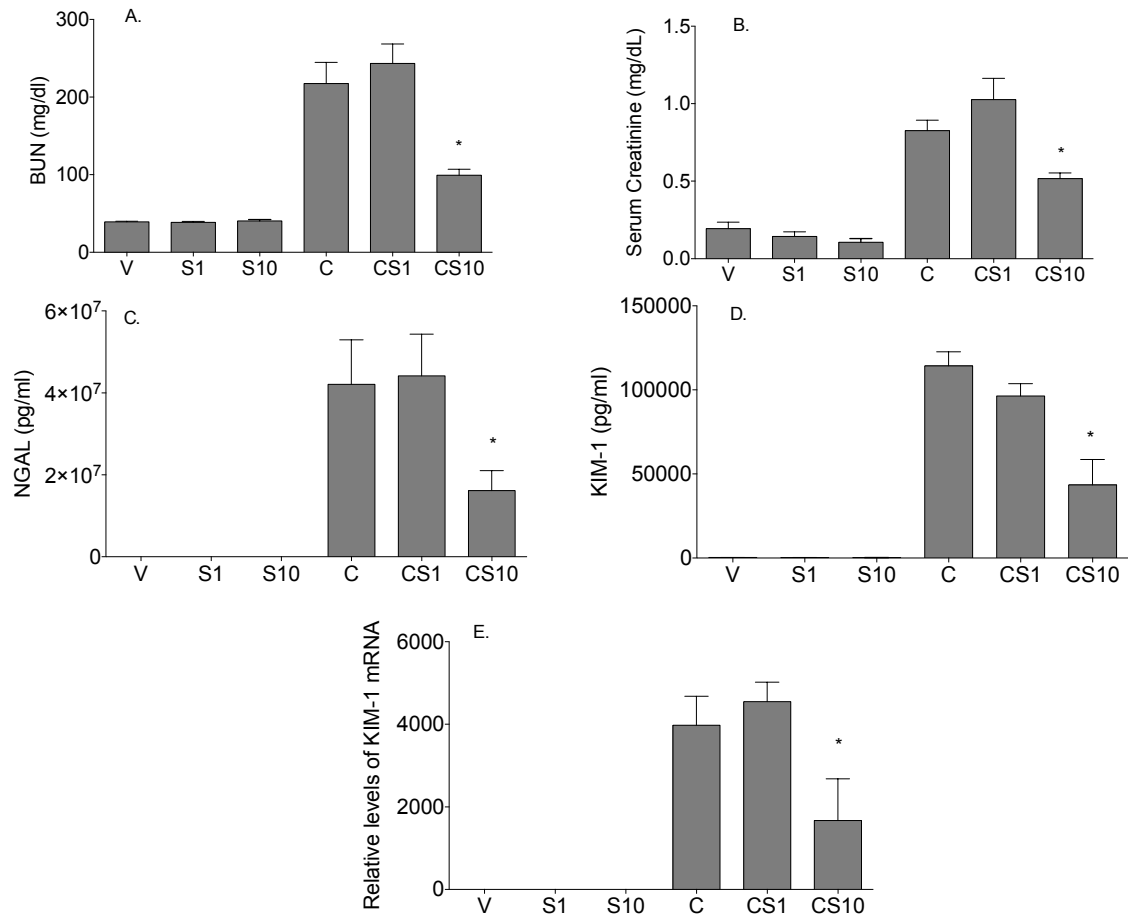
### **Statistical Analysis Data**

Data are expressed as means  $\pm$  standard error of the mean (SEM) for all the experiments. Multiple comparisons of normally distributed data were analyzed by two-way ANOVA, as appropriate, and group means were compared using Bonferroni post-tests. For western blot analysis, densitometry was performed using ImageJ software from the NIH. Data were normalized to the loading control and multiple comparisons of normally distributed data were analyzed by one-way ANOVA, as appropriate, and group means were compared using Tuckey post-tests. Single comparisons were analyzed by Student's t-test where appropriate. The criterion for statistical differences was  $p < 0.05$  for all comparisons.

## RESULTS

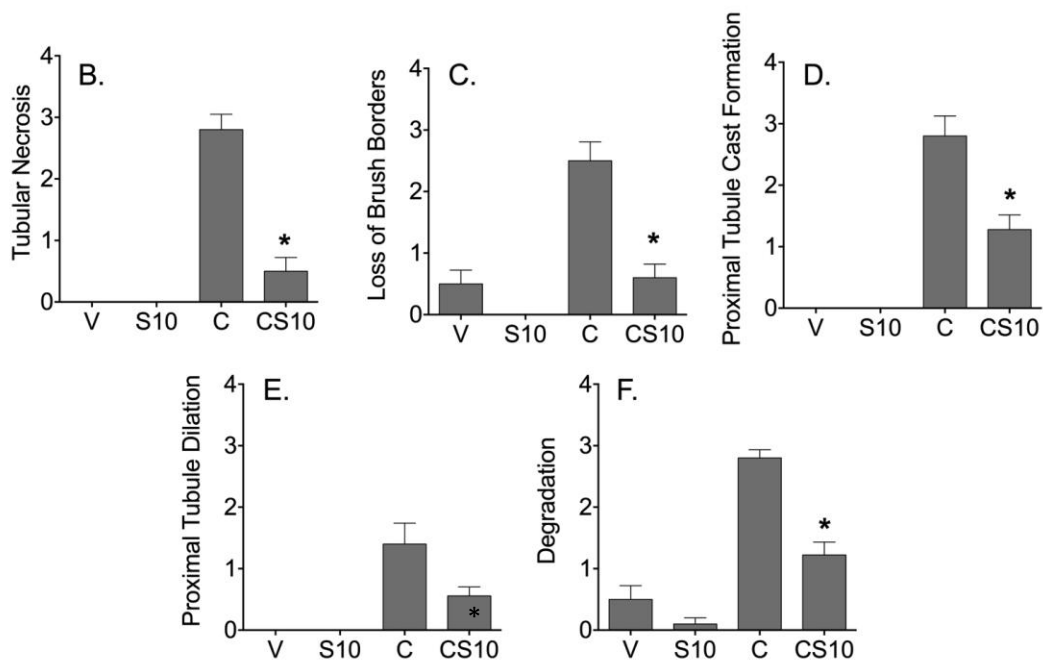
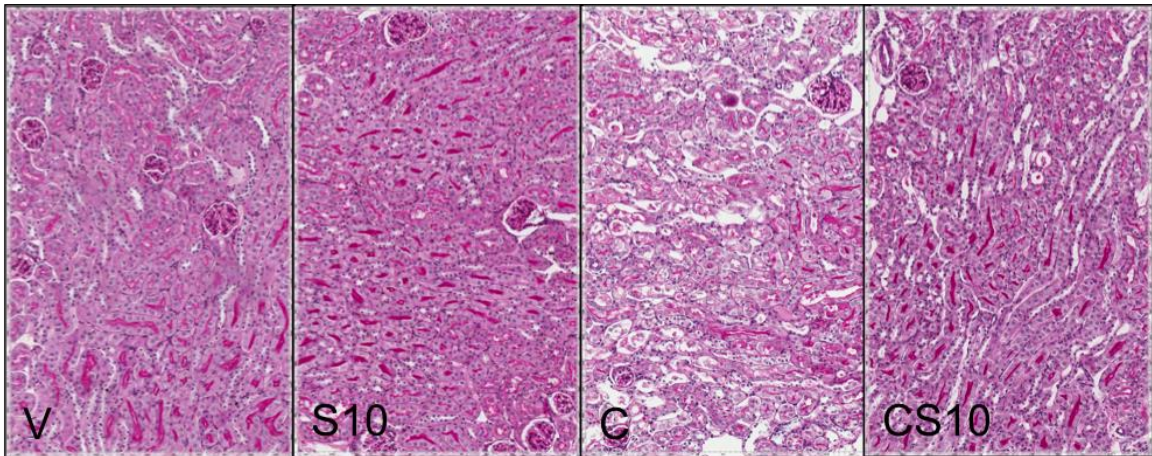
### **Effect of suramin pretreatment on serum and urine indicators of AKI in cisplatin**

**treated mice.** Suramin has been utilized in many models of kidney injury, both acute and chronic, as an agent that improves recovery from injury or fibrosis when given after disease onset (34-40); however, suramin has never before been used prophylactically to protect from kidney injury. Mice were pre-treated with suramin (1 mg/ kg and 10 mg/ kg) 72 h prior to cisplatin (20 mg/ kg) administration then sacrificed 72 h after cisplatin administration. As shown in Figure 1, cisplatin treated mice had significantly increased levels of BUN ( $217 \pm 27$  mg/ dL), serum creatinine ( $0.83 \pm 0.07$  mg/ dL ), urinary NGAL ( $4.2 \times 10^7 \pm 1.09 \times 10^7$  pg/ mL), urinary Kim-1 ( $104,050 \pm 12,429$  pg/ mL), and renal Kim-1 mRNA expression ( $3977 \pm 699$  ) as compared to vehicle treated animals (Figure 1) that indicated decreased renal function and injury. Mice pre-treated with 10 mg/ kg suramin prior to cisplatin had significantly decreased levels of BUN ( $99.27 \pm 7.67$  mg/ dL), serum creatinine ( $0.517 \pm 0.037$  mg/ dL), urinary NGAL ( $1.6 \times 10^7 \pm 4.9 \times 10^6$  pg/ mL), urinary Kim-1 ( $43,334 \pm 13,573$  pg/mL), and renal Kim-1 gene expression ( $1,669 \pm 1,013$ ); however, mice pre-treated with 1 mg/kg suramin prior to cisplatin did not show a significant decrease in the above kidney injury markers (Figure 1). These data indicate that pre-treatment of mice with 10 mg/ kg suramin is sufficient to reduce kidney injury resulting from cisplatin, while pretreatment of mice with 1 mg/ kg suramin did not.



**Figure 1. Pretreatment with high dose suramin improves markers of kidney function and injury.** C57BL/6j mice were pre-treated with suramin (1mg/ kg, S1 or 10 mg/ kg, S10) or vehicle control via i.v. through the tail vein 72 h prior to cisplatin administration (20 mg/ kg, i.p.) and mice were sacrificed 72 h after cisplatin administration. Levels of (A) blood urea nitrogen (BUN) and (B) serum creatinine were assessed via colorimetric assay and enzymatic assays, respectively. Urinary (C) NGAL and (D) Kim-1 levels were assessed via an ELISA assay. mRNA expression of (E) Kim-1 in the kidney cortex was assessed via real time qRT-PCR. n = 10; data are the mean  $\pm$  the SEM. \* indicates  $p \leq 0.05$  as compared to cisplatin treated group as determined by a two-way ANOVA. V indicates vehicle control, S1 indicates suramin i.v. pre-treatment at 1mg / kg, S10 indicates suramin i.v. pre-treatment at 10mg / kg, C indicates cisplatin i.p. at 20 mg / kg, CS1 indicates Suramin i.v. pre-treatment at 1 mg / kg followed by cisplatin i.p. at 20 mg/ kg 72 h later, CS10 indicates Suramin i.v. pre-treatment at 10 mg / kg followed by cisplatin i.p. at 20 mg/ kg 72 h later.

**Effect of suramin pretreatment on pathological damage to proximal tubules in cisplatin treated mice.** In order to determine if the kidney injury markers measured in Figure 1 were associated with renal histological changes, we assessed renal histology on hematoxylin and eosin (H&E) and periodic acid schiff (PAS) stained paraffin embedded sections collected 72 h after cisplatin administration. A renal pathologist blindly scored the following markers of proximal tubule histological damage as described in the methods: (B) acute tubular necrosis, (C) loss of brush border, (D) proximal tubular cast formation, (E) tubule dilation, and (F) degeneration. As indicated in Figure 2, all of the above markers were significantly increased in cisplatin treated mice. On the contrary, these histological changes were significantly attenuated in the mice pretreated with suramin at 10 mg/ kg (Figure 2). Thus, suramin protected mice from histological damage resulting from cisplatin treatment.



**Figure 2. Suramin prevents cisplatin-induced deterioration in kidney pathology.** Renal histological changes were assessed on H&E and PAS stained sections (5 $\mu$ M) thick. (A) Representative images of renal histology at 200X magnification. (B) Tubular necrosis, (C) loss of proximal tubule brush borders, (D) proximal tubule cast formation, (E) proximal tubule dilatation, and (F) degradation were assessed as markers of histological changes. For figures (B-F), scoring of the sections was performed in a blinded manner by renal pathologist Dr. Megyesi using a scale of 0-4 (0=not present, 1=mild, 2=moderate, 3=severe, and 4=very severe renal histological changes in the proximal tubules). n = 10; data are the mean  $\pm$  the SEM. \* indicates  $p \leq 0.05$  as compared to cisplatin treated group as determined by a two-way ANOVA. V indicates vehicle control, S10 indicates suramin i.v. pre-treatment at 10 mg / kg, C indicates cisplatin i.p. at 20 mg / kg, CS10 indicates Suramin i.v. pre-treatment at 10 mg / kg followed by cisplatin i.p. at 20 mg/ kg 72 h later.



**Effect of suramin pretreatment on inflammatory cytokines and chemokines in**

**mice treated with cisplatin.** Treatment with cisplatin has been shown to induce the

expression of many pro-inflammatory cytokines in the kidney (12, 28, 29). In Figure 3,

we assessed mRNA expression of the following pro-inflammatory cytokines and

chemokines such as: (a) Tnf- $\alpha$ , (b) Il6, (c) Cxcl-1, (d) Mcp-1, and (e) Il1 $\beta$ . All of the

aforementioned cytokines and chemokines were significantly elevated  $10.8 \pm 2.7$ ,  $66 \pm$

$10$ ,  $144 \pm 20$ ,  $15.2 \pm 4.5$ , and  $3.41 \pm 0.60$ -fold respectively over the vehicle treated

control mice with 20 mg/ kg cisplatin treatment; however, mice pre-treated with suramin

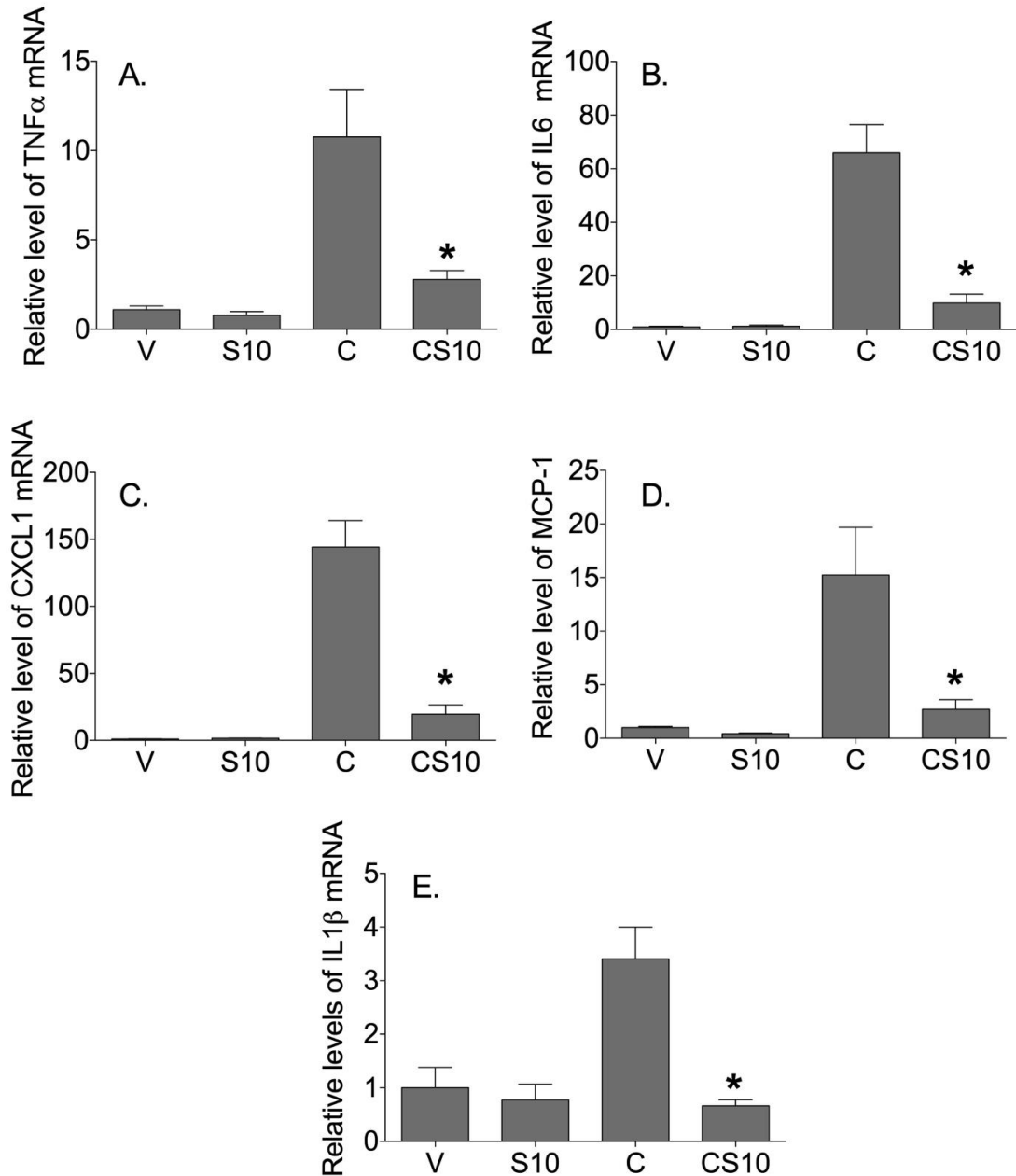
(10 mg/ kg) 72 h prior to cisplatin treatment had significantly reduced mRNA expression

$2.8 \pm 0.5$ ,  $9.8 \pm 3.4$ ,  $19.5 \pm 6.9$ ,  $2.7 \pm 0.9$ , and  $0.7 \pm 0.1$  of these pro-inflammatory

cytokines and chemokines, respectively (Figure 3). These data demonstrated that

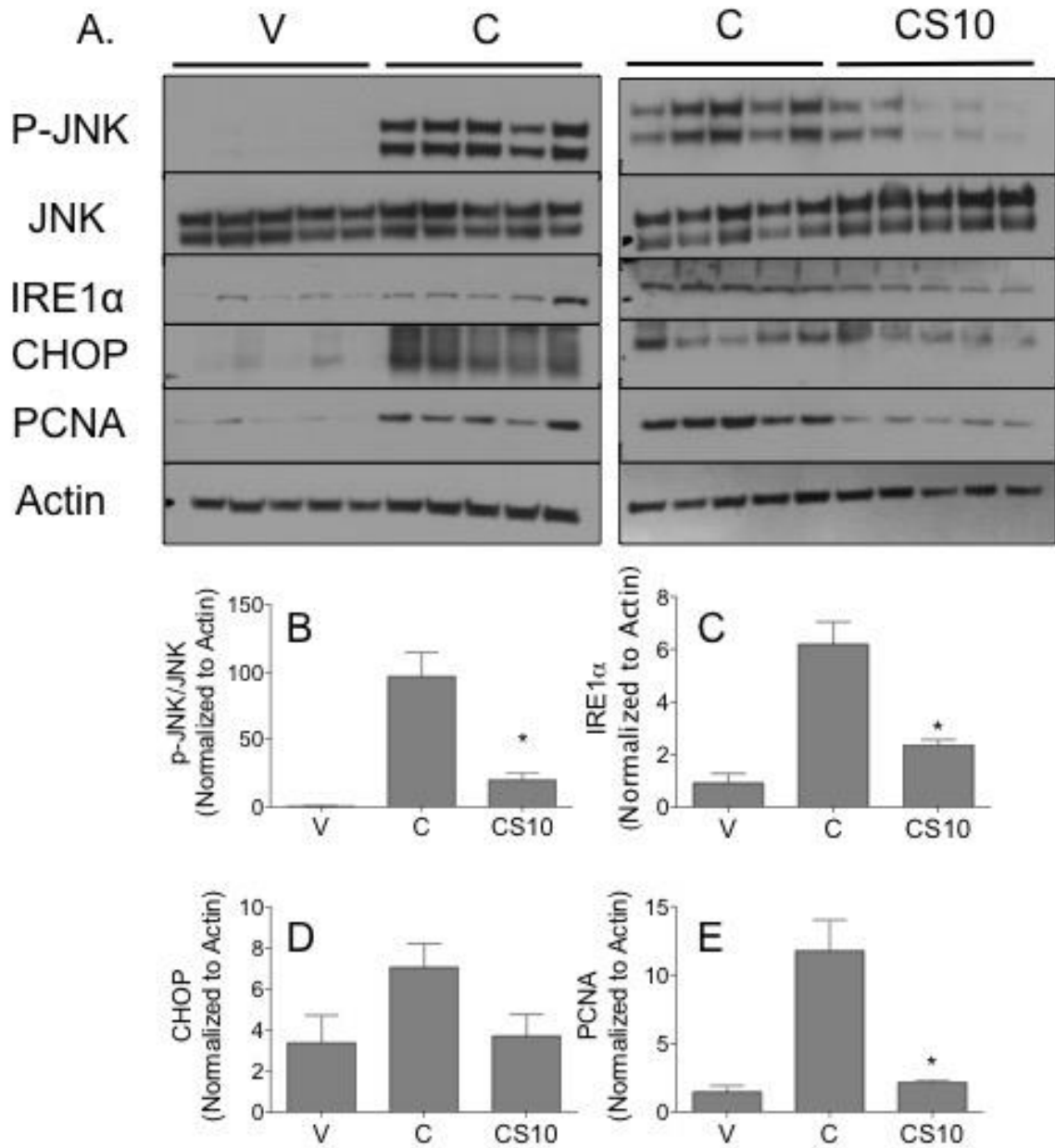
pretreatment of mice with suramin reduces cisplatin-induced expression of pro-

inflammatory cytokines and chemokines in the kidney.



**Figure 3. Suramin pre-treatment reduces inflammation in the kidney following cisplatin treatment.** C57BL/6j mice were pre-treated with suramin (1 mg/kg or 10 mg/kg or equal volume vehicle) via tail vein injection 72 h prior to cisplatin administration (20mg/kg, i.p.). Mice were sacrificed 72 h after cisplatin administration and relative expression of (A) Tnf $\alpha$ , (B) Il6, (C) Cxcl1, (D) Mcp1, and (E) Il1 $\beta$  were measured via real-time qRT-PCR. data are the mean  $\pm$  the SEM. \* indicates  $p \leq 0.05$  as compared to cisplatin treated group as determined by a two-way ANOVA. For all panels, V indicates vehicle control, S1 indicates suramin i.v. pre-treatment at 1 mg/kg, S10 indicates suramin i.v. pre-treatment at 10mg/kg, C indicates cisplatin i.p. at 20 mg/kg, CS1 indicates Suramin i.v. pre-treatment at 1 mg/kg followed by cisplatin i.p. at 20 mg/kg 72 h later, CS10 indicates Suramin i.v. pre-treatment at 10 mg / kg followed by cisplatin i.p. at 20 mg/ kg 72 h later.

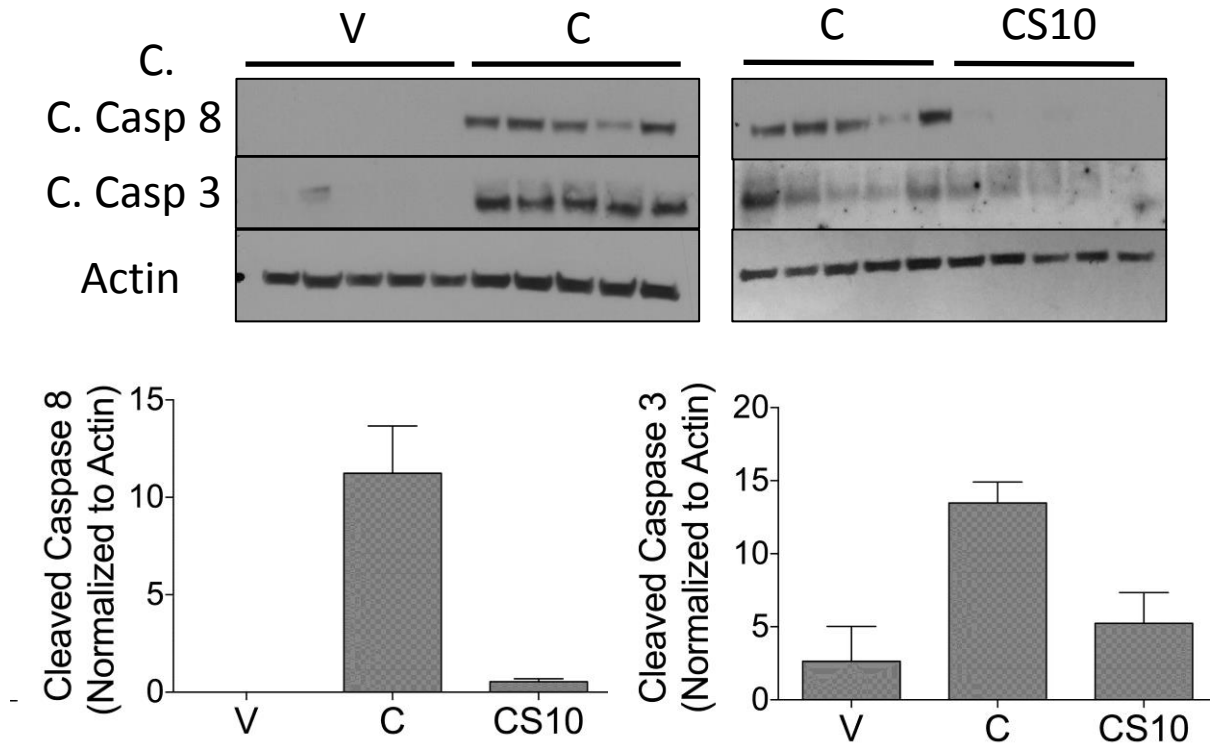
**Effect of suramin pretreatment on markers of cell stress and proliferation in cisplatin treated mice.** The kidney is known to activate a number of pathways in response to cisplatin-induced injury, including cell stress and proliferation signaling pathways (12). Thus, we assessed markers of cell stress and proliferation that are associated with cisplatin-induced AKI. Cisplatin treated mice had significantly increased levels of the activated form of pJNK ( $96.8 \pm 18.0$ ) compared to vehicle treatment, while the pretreatment with 10 mg/ kg suramin significantly decreased levels of pJNK ( $19.7 \pm 5.8$ ) compared to cisplatin treatment alone (Figure 4A and B). We also assessed markers of endoplasmic reticulum stress (ER Stress), as it has previously been implicated in cisplatin-induced AKI. Cisplatin treatment increased both IRE1 $\alpha$  ( $6.2 \pm 0.9$ ) and CHOP ( $7.1 \pm 1.2$ ) expression, while cisplatin plus suramin treatment decreased both IRE1 $\alpha$  ( $2.4 \pm 0.2$ ) and CHOP ( $3.7 \pm 1.1$ ) expression; while IRE1 $\alpha$  was significantly decreased with suramin treatment prior to cisplatin, CHOP expression exhibited a decreasing trend compared to cisplatin alone treated animals (Figure 4A and B). Finally, PCNA, a marker of proliferation, had increased expression ( $11.8 \pm 2.3$ ) with cisplatin treatment but was significantly decreased with cisplatin plus suramin treatment ( $0.5 \pm 0.2$ ). Taken together, these data indicate that suramin pre-treatment decreases cisplatin-induced increase in the levels of markers of cell stress and proliferation in the kidney cortex.



**Figure 4. Suramin prevents cisplatin-induced increases in markers of cell stress responses and proliferation.** Western blot analysis was performed to assess relative protein levels of the indicated proteins in the renal cortex of vehicle treated mice (V), cisplatin treated mice (C), and suramin pre-treated (10 mg/ kg i.v.) mice that were given cisplatin (20 mg/ kg) 72 h later (CS10). Samples from the kidney cortex were prepared from mice sacrificed 72 hours after cisplatin administration. For B-E, densitometry was performed to assess relative protein levels normalized to actin. For B-E, data are the mean  $\pm$  SEM and \* indicates statistically significant difference as compared to cisplatin treated group as determined by one-way ANOVA. n = 5

**Effect of suramin pretreatment on markers of apoptosis in cisplatin treated mice.**

Cisplatin treatment is known to induce apoptosis in the kidney (12). Thus, we assessed markers of apoptosis in the kidney cortex. Apoptosis is dependent on activation and cleavage of cysteine and aspartate proteases (caspases). In Figure 5 (A-C), western blot and the corresponding densitometry analysis of kidney cortex protein homogenates show that cisplatin treatment of mice caused  $11.2 \pm 2.4$  and  $13.4 \pm 1.4$  -fold increases in the levels of cleaved caspase 8 and cleaved caspase 3, respectively. Suramin plus cisplatin treated mice had significantly reduced levels of cleaved caspases 8 and 3 in the kidney cortex, with  $0.5 \pm 0.2$  and  $5.2 \pm 2.1$ , respectively. These data indicate that suramin pre-treatment protects from cisplatin-induced kidney cell apoptosis.



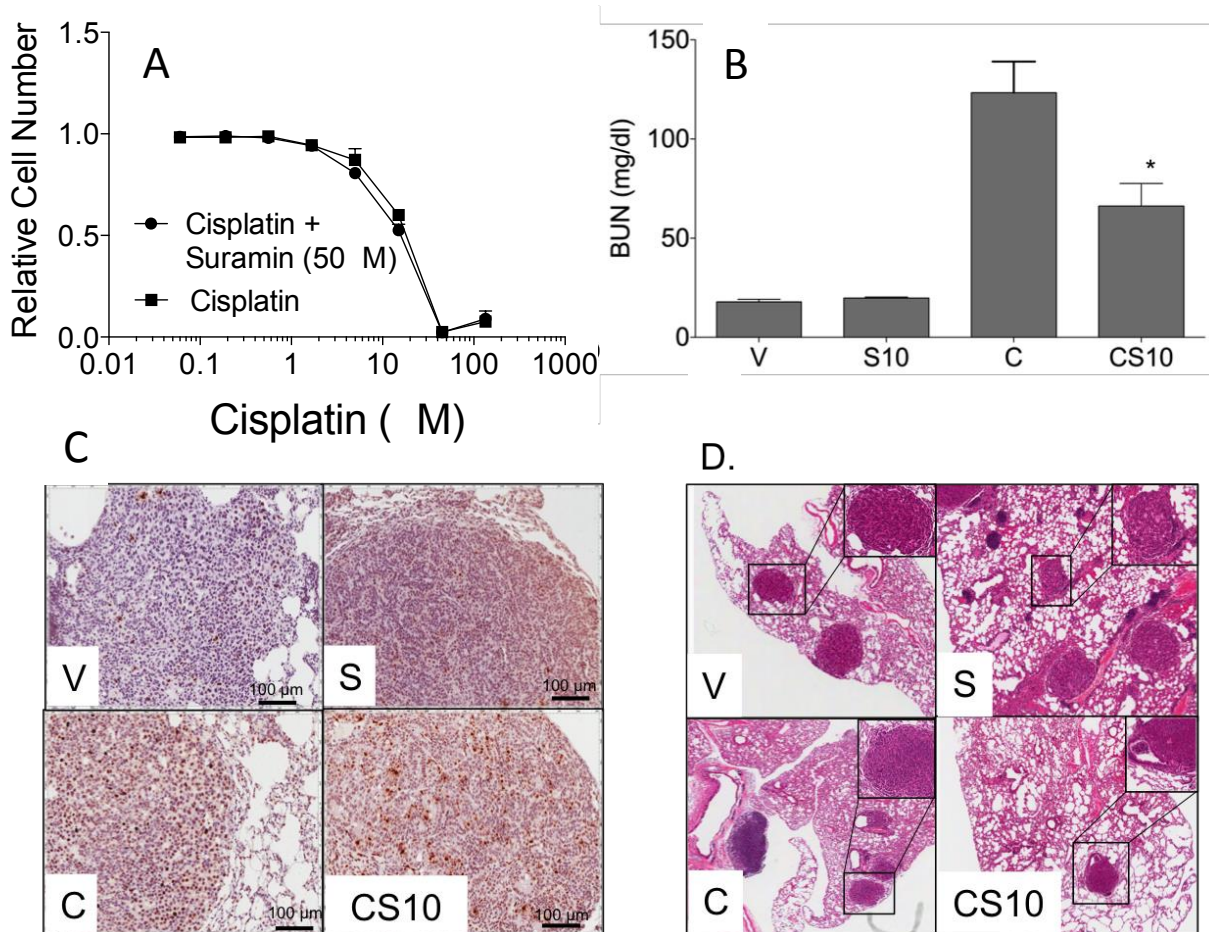
**Figure 5. Pretreatment with suramin inhibits cisplatin-induced cell death in the kidney.** C57BL/6j mice were pre-treated with suramin (10 mg / kg, i.v.) or vehicle control 72 h prior to cisplatin (20 mg / kg, i.p.) and mice were sacrificed after an additional 72 h. (A) Western blot analysis of cleaved caspase 8 and cleaved caspase 3 from homogenates of kidney cortex for the following treatment groups: Vehicle (V), cisplatin (C), Cisplatin +Suramin 10 mg/kg (CS10). For B and C, densitometry was performed to assess relative protein levels normalized to actin. For B and C data are the mean  $\pm$  SEM and \* indicates statistically significant difference as compared to cisplatin treated group as determined by one-way ANOVA.

### **Effect of suramin pre-treatment and cisplatin on mutant Kras lung cancer mice.**

Cisplatin-induced reduction in tumor mass is dependent on its ability to induce DNA damage and apoptosis. If suramin is to be utilized with cisplatin as part of a treatment protocol, then it cannot directly reduce cisplatin-induced apoptosis in cancer cells. We assessed the effects of suramin on the tumoricidal activity of cisplatin both *in vitro* and *in vivo*. A549 cells, a non-small cell lung cancer cell line expressing mutant KRAS, were pre-treated with 50  $\mu$ M suramin for 1 h, then treated with cisplatin (0-135  $\mu$ M, 48 h) (Figure 6A). We chose to pretreat A549 cells with 50  $\mu$ M because 50  $\mu$ M suramin is known to induce renal proximal tubule cell proliferation and scattering (42). Thus 50  $\mu$ M suramin is a concentration that may provide renoprotective effects against cisplatin-induced kidney injury. There was no difference in the dose response curves of the cisplatin-treated A549 cells with or without 50  $\mu$ M suramin pre-treatment. To examine the *in vivo* effects of suramin, FVB mice expressing doxycycline-inducible mutant Kras driven lung adenocarcinoma were treated with cisplatin alone or pretreated with suramin 72 h prior to cisplatin. Levels of BUN were measured as a marker of kidney function. BUN levels were significantly higher in mice treated with cisplatin compared to mice pre-treated with suramin (10 mg/ kg) 72 h prior to cisplatin (Figure 6B). The ability of cisplatin to induce DNA damage in the lung adenocarcinomas in the presence and absence of suramin was evaluated by performing immunohistochemistry of  $\gamma$ -H2A.x staining in lung sections. Studies have found an association between  $\gamma$ -H2A.x activation, and reduced cell viability (43, 44). In Figure 6C, we observed significant increase in  $\gamma$ -H2A.x staining in the lung nodules of cisplatin treated mice compared to vehicle treated animals. The lung nodules from mice receiving suramin pre-treatment prior to cisplatin showed no difference in  $\gamma$ -H2A.x staining compared to the staining in cisplatin only treated mice (Figure 6C). Figure 6D depicts H&E stained lung sections for the visualization of the tumor surrounded by adjacent normal tissue. Taken together, these data suggest that

suramin pre-treatment protects mice from cisplatin-induced AKI without inhibiting the ability of cisplatin to induce DNA damage in lung adenocarcinomas.





**Figure 6. Suramin treatment does not prevent the anti-tumor efficacy of cisplatin.** (A) A549 cells were treated with 0-135  $\mu$ M cisplatin in the presence and absence of 1  $\mu$ M suramin for 24 h. Cell viability was assessed via Alamar Blue assay. (B) BUN levels of 10-month old FVB mice harboring mutant Kras driven lung tumors treated with 10 mg/kg suramin 72 h prior to cisplatin treatment (20 mg/kg). Mice were sacrificed 48h after cisplatin administration. Immunohistochemistry of (C)  $\gamma$ H2A.x and (D) H&E Stained lung sections from mice in B. V indicates vehicle control, S1 indicates suramin i.v. pre-treatment at 1 mg/kg, S10 indicates suramin i.v. pre-treatment at 10mg/kg, C indicates cisplatin i.p. at 20 mg/kg, CS1 indicates Suramin i.v. pre-treatment at 1 mg/kg followed by cisplatin i.p. at 20 mg/kg 72 h later, CS10 indicates Suramin i.v. pre-treatment at 10 mg/kg followed by cisplatin i.p. at 20 mg/ kg 72 h later. \* indicates statistically significant difference as compared to cisplatin treated group as determined by two-way ANOVA. n = 5 - 10

## DISCUSSION

Previous studies have shown that treatment with suramin after the onset of kidney injury accelerates recovery from injury (35, 38, 40); however, suramin has never before been used prophylactically to protect from kidney injury. The use of suramin in a model of cisplatin-induced AKI also has never before been studied. Thus, in this study we focus on the prophylactic use of suramin as a renoprotective agent against the pathogenesis of cisplatin-induced AKI. Our results indicate that suramin protects mice from cisplatin-induced AKI at 10 mg/kg, but not at a lower dose of 1 mg/kg. Markers of kidney function and injury were improved in mice treated with the higher dose of suramin. Data indicated that suramin attenuated the inflammatory response associated with cisplatin-induced AKI, and also with decreased markers of cell stress and proliferation in the renal cortex following cisplatin administration. Suramin also decreased cisplatin-induced induced death receptor and/ or intrinsic apoptosis as evidenced by lower levels of cleaved caspases 8 and 3 in the kidney cortex. Interestingly, our *in vitro* data suggested that the protection observed *in vivo* may not be mediated by suramin directly blocking kidney cell death as BMK cells pretreated with 1  $\mu$ M suramin were not protected from cisplatin-induced reduction in cell numbers. Likewise, suramin did not attenuate reduced cell viability in A549 lung cancer cells treated with cisplatin. Finally, suramin pre-treatment (10 mg/kg) of FVB mice harboring mutant Kras driven lung adenocarcinomas were protected from cisplatin-induced AKI as evidenced by improved BUN. However, suramin did not block the ability of cisplatin to induce DNA damage in the lung adenocarcinomas. Taken together, our data suggest that suramin pre-treatment prior to cisplatin administration protects the kidney from

cisplatin-induced AKI without inhibiting cisplatin's cytotoxic activity. These data suggest that suramin may be efficacious for widening the therapeutic window of cisplatin for the treatment of lung cancer.

Suramin has been shown to attenuate the inflammatory response associated with the disease progression in multiple models of both AKI and chronic kidney disease (CKD) (34, 35, 37, 38). Interestingly, suramin is a pan purinergic P2 receptor antagonist, and P2 receptors are known to play a regulatory role in the mounting of an inflammatory response following tissue injury (30, 31, 45). A myriad of P2 receptors are expressed on both the proximal tubule cells (32, 46) and leukocytes (30) involved in the pathogenesis of cisplatin-induced AKI. P2 receptors are known to play a regulatory role in the release of cytokines and chemokines such as MCP-1 and CXCL-1 (47-49). Interestingly, in our studies, we found that expression of both MCP-1 and CXCL-1, both of which are pro-inflammatory chemokines and cytokines respectively, are decreased following cisplatin treatment in mice that were pre-treated with suramin. Our data indicate that suramin pre-treatment also decreases the renal expression of TNF $\alpha$ , IL-1 $\beta$ , and IL-6 mRNA. Mast cells have been implicated in the pathogenesis of cisplatin-induced AKI and are known to release pro-inflammatory cytokines upon degranulation including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 (12, 50). Suramin has been shown to inhibit mast cell degranulation (51), which may be a mechanism by which it inhibits expression of these cytokines that are known to perpetuate kidney injury.

It has been shown that during cisplatin-induced AKI, TNF- $\alpha$  signals through TNF- $\alpha$  receptor (TNFR) for the activation of many cell stress and cell death pathways during cisplatin-induced AKI (10, 12, 26, 27). TNF- $\alpha$  signaling through TNFR has been shown to induce phosphorylation of JNK, which participates in ER stress and apoptosis (52-54). ER stress-induced apoptosis plays a role in cisplatin-induced AKI (5). During cisplatin-induced AKI, renal cells are under stress as a result of increased cellular damage, which

leads to activation of ER stress-mediated pathways. IRE1 $\alpha$  is activated during ER stress and signals through JNK for the cells to undergo ER stress-induced apoptosis (53, 55). Our results indicate that cisplatin treatment increases IRE1 $\alpha$  and JNK activation, while pre-treatment with suramin decreases cisplatin-induced IRE1 $\alpha$  expression and pJNK. Interestingly, this is not the only pathway of ER stress that mediates apoptosis. CHOP is a transcription factor, which is associated with activating ER stress-induced apoptosis independently of IRE1 $\alpha$  and JNK (55). We found that CHOP expression was significantly decreased with pre-treatment of suramin prior to cisplatin. These results suggest that suramin protects from ER stress-induced apoptosis; however, there are many other pathways of apoptosis implicated in cisplatin-induced AKI.

Cisplatin administration has been shown to induce many pathways of cell death including both apoptosis and necrosis (5, 10, 12). During cisplatin-induced AKI, many pathways of apoptosis are activated, including the death receptor mediated pathway of apoptosis (5, 10, 12). In this pathway of apoptosis, TNF- $\alpha$  or other death receptor ligands can activate death receptors on the proximal tubule cell surface leading to caspase 8 cleavage and activation followed by the cleavage and activation of downstream effector caspases such as caspase 3, leading to apoptosis (52). Suramin has been shown to inhibit TNF- $\alpha$  from binding with the death receptor (33). Interestingly our results show that pre-treatment with suramin prior to cisplatin treatment significantly decreases both caspase 8 and caspase 3 cleavage, suggesting that suramin is preventing cisplatin-induced death receptor-mediated apoptosis in the kidney, while suramin pretreatment also decreased the renal expression of TNF- $\alpha$  in mice treated with cisplatin. On the contrary, we found that suramin does not inhibit cell death induced directly by cisplatin in a lung cancer cell line. Thus, our *in vivo* and *in vitro* data suggest that suramin protects from cisplatin-induced AKI through the attenuation of the inflammatory response.

More importantly, suramin is currently being pursued for the treatment of many cancers as a single agent and in combination therapy with many anti-cancer chemotherapeutic agents including cisplatin (56-59). Our data also indicate that suramin does not inhibit cisplatin's cytotoxic efficacy. These data indicate that the use of suramin to attenuate cisplatin-induced AKI has great translational potential. In future studies, it will be important to determine if suramin post-treatment following cisplatin is sufficient to speed recovery from cisplatin-induced AKI. Elucidating an optimal dosing regimen for the use of suramin in the treatment/prevention of cisplatin-induced AKI will be important clinically.

## SUMMARY

Cisplatin, a commonly used chemotherapeutic in the treatment of many solid tumor cancers has a dose limiting side effect of nephrotoxicity. There are currently no treatment options for AKI besides palliative care. Thus, there is a clear need for the development of renoprotective strategies in the treatment/prevention of cisplatin-induced AKI. In this study, we utilized an FDA approved drug, suramin, which has been shown to speed recovery from multiple models of kidney injury encompassing both acute and chronic kidney disease.

It was observed that pretreatment with suramin at a high dose (10 mg/ kg) prior to cisplatin treatment protected from cisplatin-induced AKI, while a low dose of suramin (1 mg/ kg) did not exhibit renoprotective effects against cisplatin-induced AKI. Pre-treatment with suramin at 10 mg/ kg prior to cisplatin treatment attenuated the gene expression of pro-inflammatory cytokine mRNAs associated with the pathogenesis of cisplatin-induced AKI. It was also found that suramin pre-treatment prior to cisplatin treatment decreased markers of cell stress and proliferation. Suramin pre-treatment also decreased death receptor mediated apoptosis following cisplatin administration. *In vitro* experiments indicated that suramin pre-treatment does not protect from cell death induced directly by cisplatin in kidney cells, suggesting that suramin's renoprotective effects are most likely through the attenuation of the inflammatory response. Finally, suramin pre-treatment prior to cisplatin administration protected mice harboring mutant Kras-driven lung tumors from cisplatin-induced kidney injury, while not inhibiting cisplatin's tumoricidal efficacy.

In conclusion, the data obtained from this study indicate that suramin has great potential to be repurposed as a renoprotective agent to protect patients from cisplatin-induced AKI. Thus, the use of suramin as a renoprotective agent will potentially widen the therapeutic window of cisplatin, enabling patients to receive more effective treatment of their cancer, which will hopefully improve their prognosis and quality of life while receiving cisplatin treatment.

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## LIST OF ABBREVIATIONS

AKI	Acute Kidney Injury
ANOVA	Analysis of Variance
BUN	Blood Urea Nitrogen
CHOP	C/EBP homologous protein
CKD	Chronic Kidney Disease
Ctr-1	copper transporter-1
CXCL-1	chemokine (C-X-C) Ligand-1
GGT	Gamma glutamyl transpeptidase
H&E	Hematoxylin and Eosin
IL-18	Interleukin- 18
IL-1 $\beta$	Interleukin- 1 $\beta$
IL-6	Interleukin- 18
IRE-1 $\alpha$	Inositol requiring enzyme-1 $\alpha$
JNK	c-Jun N-terminal Kinase
KIM-1 or HAVCR	kidney Injury Molecule-1 or Hepatitis A Virus Cellular Receptor

KRAS	Kirstin Rat Sarcoma Viral Oncogene
MATE-1	Multidrug and Toxin Extrusion-1 Transporter
MCP-1 or CCL-2	Monocyte Chemotactic Protein-1 or Chemokine (C-C) Ligand-2
NGAL	Neutrophil Gelatinase Associated Lipocalin
OCT-2	Organic Cation Transporter-1
P2 Receptors	Purinergic Type 2 Receptors
PAS	Periodic Acid Schiff
PCNA	Proliferating Cell nuclear Antigen
TNF- $\alpha$	Tumor Necrosis Factor- $\alpha$

## CURRICULUM VITAE

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### EDUCATION

2009-2012 University of Louisiana at Monroe  
B.S. Toxicology, Minor Chemistry  
GPA 3.75

### HONORS

2015 Participant in Vanderbilt's mouse model in kidney injury course  
2014 School of Medicine Travel Award  
2014 Graduate Student Council Travel Award  
2013 IPIBS Fellow in Pharmacology and Toxicology, covering tuition,  
stipend, benefits  
2011 Phi Kappa Phi Honor Society  
2009-2012 University of Louisiana at Monroe Academic Scholarship  
2009-2012 Presidential Scholar (3.95-4.0 GPA; 2 semesters)  
Dean's List (>3.5 GPA; 3 semesters)

### TEACHING AND RELATED EXPERIENCE

2015 Guest Lecturer Introduction to Pharmacology- Diuretics  
University of Louisville  
2015 Simulation Modulation Facilitator- Organophosphates  
University of Louisville  
2012 Academic Tutor- Math, Biology, Chemistry  
University of Louisiana at Monroe  
2011-2012 Supplemental Instructor- Chemistry  
University of Louisiana at Monroe  
2010-2011 Environmental Intern  
Gulf Island Fabrication

## RESEARCH

- 2013- IPIBS Graduate Fellowship  
University of Louisville
- 2013 Cancer Education Program  
University of Louisville
- 2012 Summer Undergraduate Pharmacology Experience in Research  
Louisiana State University Health Science Center-Shreveport
- 2011 Emerging Scholar  
University of Louisiana-Monroe

## PEER REVIEWED PUBLICATIONS

Zeiderman, M. R., Egger, M. E., Kimbrough, C. W., England, C. G., Dupre, T. V., McMasters, K. M., & McNally, L. R. (2014). Targeting of BRAF resistant melanoma via extracellular matrix metalloproteinase inducer receptor. *J Surg Res*, 190(1), 111-118. doi: 10.1016/j.jss.2014.02.021

## ABSTRACTS

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- Dupre, TV**, Mather, AR, and Siskind, LJ. Inhibitors of Ceramide Synthesis Protect mice from cisplatin-induced acute kidney injury. South Eastern Regional Lipid Conference, November 2014.
- Dupre, TV**, Mather, AR, and Siskind, LJ. Inhibitors of Ceramide Synthesis Protect mice from cisplatin-induced acute kidney injury. Ohio Valley Society of Toxicology, March 2014.
- Dupre, TV**, Mather, AR, and Siskind, LJ. Inhibitors of Ceramide Synthesis Protect mice from cisplatin-induced acute kidney injury. Research Louisville, September 2014.
- Dupre, TV**, Mather, AR, and Siskind, LJ. Inhibitors of Ceramide Synthesis Protect mice from cisplatin-induced acute kidney injury. Society of Toxicology, March 2014.
- Zeiderman, M, Egger, ME, England, CG, Kimbrough, CW, **Dupre, TV**, McMasters, KM, and McNally, LR. EMPRINN expression provides a novel target for cell-specific targeting of metastatic melanoma. *American Surgical Congress*, February 2014.
- Kimbrough, CW, England, CG, **Dupre, TV**, Huang, J, Hudson, SV, and McNally, LR. Syndecan-1 probe selectively localizes to pancreatic adenocarcinoma. *American Surgical Congress*, February 4, 2014.
- Dupre, TV**, England, CG, Huang, J, and McNally, LR. Detection of pancreatic adenocarcinoma *in vivo* with S100A9 liposomes. *Research Louisville*, Louisville, KY, September 2013.
- Huang, J, England, CG, Wenyuan, Y, **Dupre, TV**, Sabrin, A, Rush, J, Ceresa, BP, Frieboes, H, McNally, LR. EGFR Targeting of Pancreatic Cancer using

Multispectral Optoacoustic Technology. Research Louisville, Louisville, KY.  
September 2013.

Hudson, SV, Kimbrough, CW, Huang, J, **Dupre, TV**, England, CG, and McNally, LR.  
Optimal Imaging of Hypoxia in Pancreatic Adenocarcinoma. *Research Louisville*,  
Louisville, KY September 24, 2013.

Pickelsimer, DD, **Dupre, TV**, Huang, J, Kimbrough, CW, Hudson, SV, England, CG, and  
McNally, LR. Pancreatic Adenocarcinoma Detected with Targeted Liposomes.  
Research Louisville, Louisville, KY. September 2013.

Zeiderman, M, Egger, M, Kimbrough, CW, England, CG, **Dupre, TV**, McMasters, K, and  
McNally, LR. Novel Therapies for BRAF-Inhibitor Resistant Melanoma. Research  
Louisville, Louisville, KY, September 2013.

Kimbrough, CW, **Dupre, TV**, England, CG, Huang, J, and McNally, LR. Pancreatic  
Adenocarcinoma detected by Multispectral Optoacoustic Tomography using  
Syndecan-1 probe. Research Louisville, Louisville, KY, September 2013.

**Dupre, TV**, Landry, GM, and McMartin, KE. Diglycolic Acid, the Nephrotoxic Metabolite  
of Diethylene Glycol, Inhibits Intracellular <sup>14</sup>C-succinate Uptake in Human  
Proximal Tubule Cells *in vitro*. Society of Toxicology South Central Chapter.  
October 2012.

#### PRESENTATIONS

Suramin protects from cisplatin-induced acute kidney injury. Ohio Valley Society of  
Toxicology- Student Meeting, June 2014

#### MEMBERSHIPS

Society of Toxicology  
American Society for Pharmaceutical and Experimental Therapeutics  
Ohio Valley Society of Toxicology  
Phi Kappa Phi National Honor Society