# Chronopharmacology studies in a clinically-relevant mouse model of cisplatin nephrotoxicity


Olivia N. Jacobs[1], Cierra Sharp[2], Mark A. Doll[2], Douglas Saforo[2], Tess V. Dupre[2], Levi J. Beverly[3], and Leah J. Siskind[2]

1 Department of Chemistry, University of Louisville, Louisville, KY

2 Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY

3 Department of Medicine, University of Louisville, Louisville, KY


## Abstract


Background: Cisplatin (CDDP) is a potent chemotherapeutic, but its dose-limiting side effect is nephrotoxicity which occurs in 30% of patients treated with the drug. The overall nephrotoxicity of CDDP is determined by many factors, including the amount of CDDP given and how frequently the drug is given. Nephrotoxicity is also determined by the time of day at which cisplatin is administered to patients- a field of study known as chronopharmacology. Clinical trials have shown that treating patients at different times of the day can lead to differences in level and type of injury sustained. However, the differences in injury have not been fully explored experimentally.

Methods: To address this issue, we first treated 40 wk old male and female noncancerous mice with our repeated dosing regimen of CDDP (7 mg/kg CDDP 1x/ wk for 4 wks) at either 6 a.m, 12 p.m, or 6 p.m. We then wanted to see the effects on cancerous mice, so we treated 40 wk old male and female noncancerous and cancerous mice with our repeated dosing regimen (7 mg/kg CDDP 1x/ wk for 4 wks) at either 6 a.m. or 6 p.m. Markers of kidney function, injury, and cell cycle regulation were assessed in addition to circadian gene expression.

Results: We found that *Bmal1* shows most variation with cisplatin treatment at 6am versus 6pm in both cancerous and noncancerous mice. In addition, other circadian rhythm markers *Clock, Per2, Cry2,* and *Rev-erbα* were altered with CDDP treatment, indicating CDDP dysregulates circadian function in the kidney, although overall changes in kidney injury (tubular damage and fibrosis) at the different time points examined were not drastically different.

Conclusion: We have characterized circadian expression in the kidney and lungs with a clinically relevant model of cisplatin treatment. While changes in circadian gene regulation were observed with CDDP treatment, but there is little effect on overall pathology.


## Schematic 1

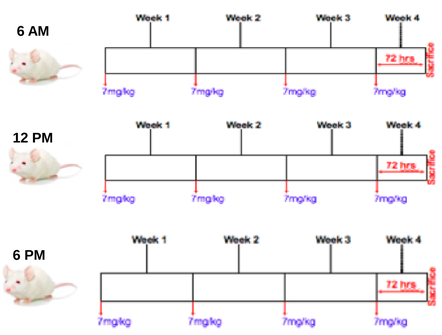



**Figure 1. Repeated dosing regimen of cisplatin.** 40 week old FVB/n mice were treated with the repeated dosing regimen of CDDP once a week for four weeks at either 6am, 12pm, or 6pm and sacrificed at day 24.

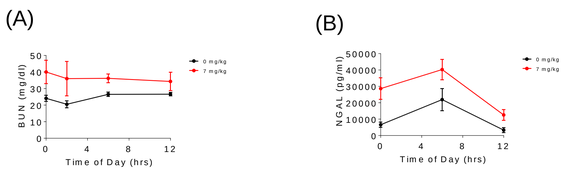



**Figure 2. Changes in kidney function and injury.** 40 wk old FVB/n mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6 am, 12 pm, or 6 pm and sacrificed at day 24. (A) Blood urea nitrogen (BUN) was measured in plasma. (B) Neutrophil gelatinase-associated lipocalin (NGAL) was measured in urine at Day 24. Time=0 denotes 6 a.m. time point.

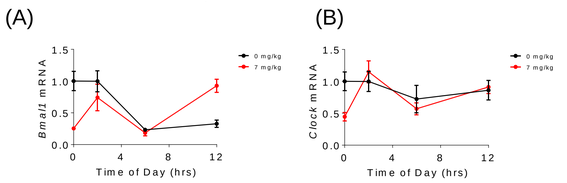



**Figure 3. Expression of core clock proteins.** 40 wk old FVB/n mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6 am, 12 pm, or 6 pm and sacrificed at day 24. Levels of core clock gene expression levels (A) BMAL1(ARNTL), and (B) CLOCK were measured in the kidney via QRTPCR.

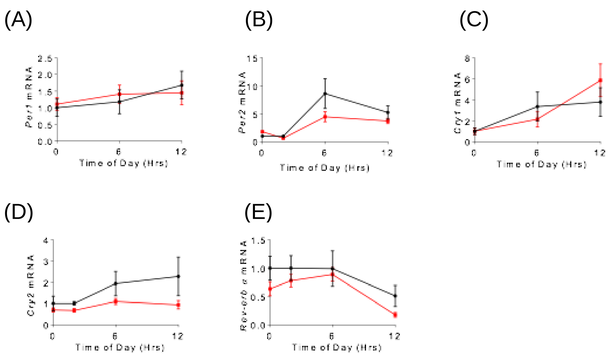



**Figure 4. Expression of clock regulated genes.** 40 wk old FVB/n mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6 am, 12 pm, or 6 pm and sacrificed at day 24. Circadian markers (A)*Per1, (B) Per2,* (C) *Cry1,* (D) *Cry2,* and (E) *Reverbα* were measured in kidney cortex via QRTPCR.

## Results

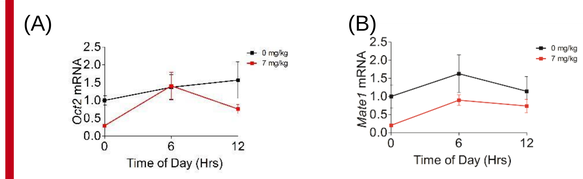



**Figure 5. Expression levels of transporters involved in uptake/extrusion of CDDP.** 40 wk old FVB/n mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6 am, 12 pm, or 6 pm and sacrificed at day 24. (A) Organic Cation Transporter 2 (OCT2) and (B) Multidrug and Toxin Extrusion 1(MATE1) levels were measured in kidney cortex via QRTPCR.

| INJURY INDICES | 6:00 AM | 12:00 PM | 6:00 PM |
|---|---|---|---|
| TNFA | INCREASE | NO CHANGE | INCREASE |
| IL6 | INCREASE | INCREASE | INCREASE |
| MCP-1 | INCREASE | INCREASE | INCREASE |
| INOS | NO CHANGE | NO CHANGE | NO CHANGE |
| ARG-1 | INCREASE | INCREASE | NO CHANGE |
| F4/80 | INCREASE | INCREASE | INCREASE |
| NECROSIS | 1 | 1 | 0.5 |
| LOSS OF BRUSH BORDER | 2 | 1 | 1 |
| TUBULAR CASTS | 2 | 1.5 | 2 |
| TUBULAR DILATION | 2 | 1 | 2 |
| TUBULAR DEGENERATION | 2 | 1 | 1 |
| TUBULAR REGENERATION | 1 | 2 | 0 |
| IMMUNE CELL INFILTRATION | 1.5 | 2 | 2.5 |
| FIBROSIS | 1 | 2 | 2.5 |

**Figure 6. Summary of injury at all time points.** A summary of the degree of injury induced by CDDP at 6AM,12PM, and 6PM. For tubular damage and fibrosis, H&E and PAS stained kidney sections were scored by a mouse renal pathologist for severity on a scale of 1-4.

## Schematic 2

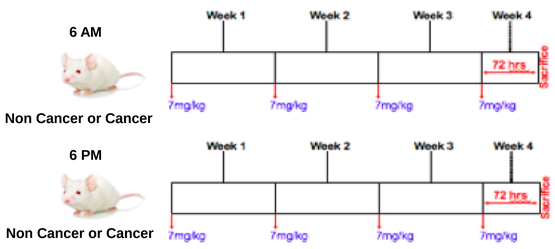



**Figure 7. Repeated dosing regimen of cisplatin.** 40 week old FVB/n mice with and without cancer were treated with the repeated dosing regimen of CDDP once a week for four weeks at either 6am or 6 pm and sacrificed at day 24.

## Kras4bG12D Transgenic Model of Lung Adenocarcinoma

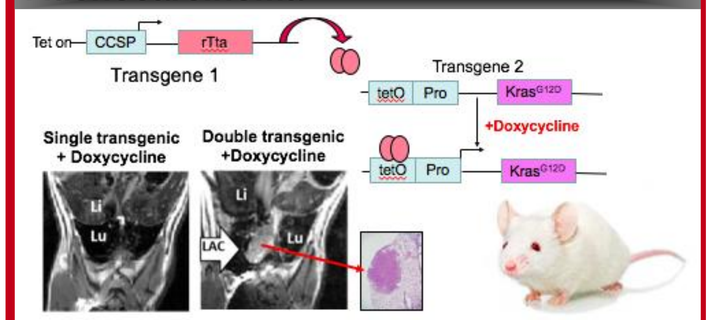



**Figure 8. Inducible, transgenic model of mutant Kras lung adenocarcinoma.** In this double transgenic model, the reverse tetracycline transactivator(rtTA) is constitutively expressed in type II lung epithelial cells, but can only activate expression of mutant Kras in the presence of doxycycline.

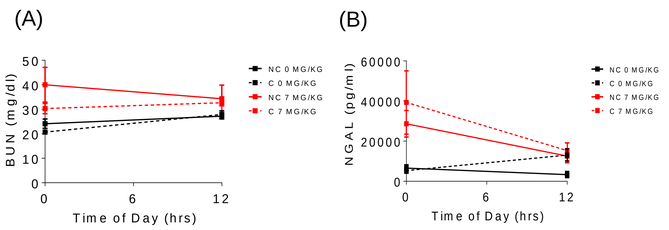



**Figure 9. Changes in kidney function and injury.** 40 wk old cancerous and noncancerous mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6AM or 6PM and sacrificed at Day 24. (A) Blood urea nitrogen (BUN) was measured in plasma. (B) Neutrophil gelatinase-associated lipocalin (NGAL) was measured in urine at Day 24. Time=0 denotes 6 a.m. time point.

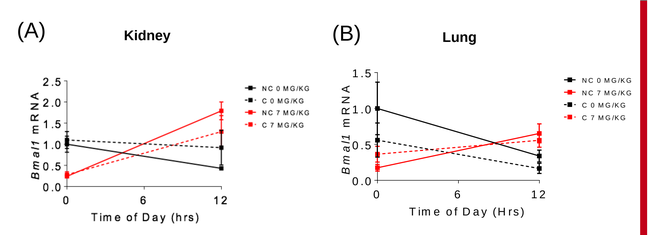
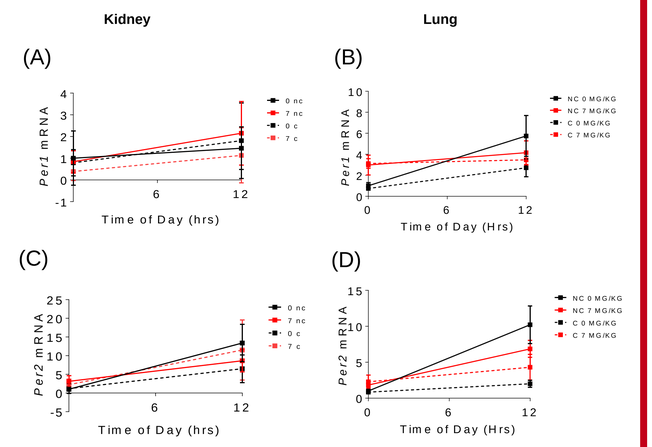
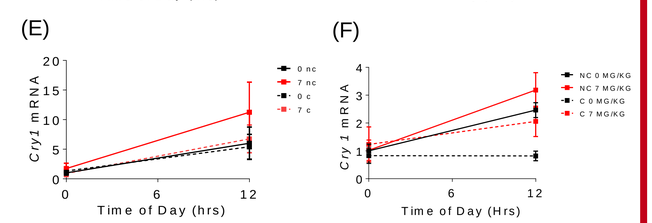



**Figure 10. Expression of Core Clock Proteins.** 40 wk old cancerous and noncancerous mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6AM or 6PM and sacrificed at Day 24. Levels of BMAL1 (ARNTL) were measured in kidneys (A) and lung (B). Levels of CLOCK were measured in kidneys (C) and lung (D) via QRTPCR.



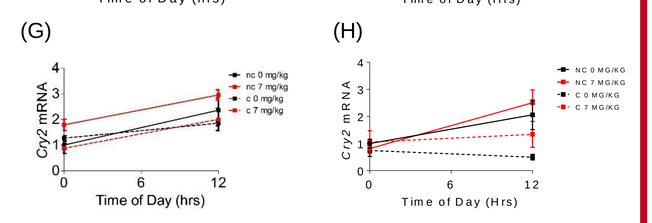




**Figure 11. Expression of clock regulated proteins.** 40 wk old cancerous and noncancerous mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6AM or 6PM and sacrificed at Day 24. Circadian markers (A,B)*Per1,* (C,D*) Per2,* (E,F) *Cry1,* (G,H) *Cry2,* and (I,J) *Reverbα* were measured in the kidney and lung via QRTPCR.

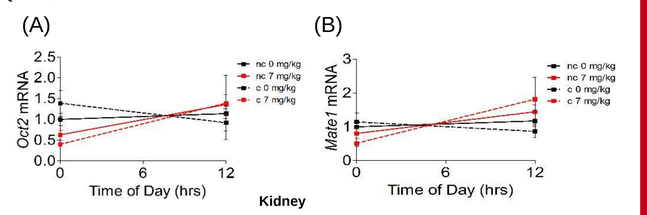



**Figure 12. Expression levels of transporters involved in uptake/extrusion of CDDP.** 40 wk old cancerous and noncancerous mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6AM or 6PM and sacrificed at Day 24. (A) Organic Cation Transporter 2(OCT2) and (B) Multidrug and Toxin Extrusion 1(MATE1) levels were measured in kidney cortex via QRTPCR.

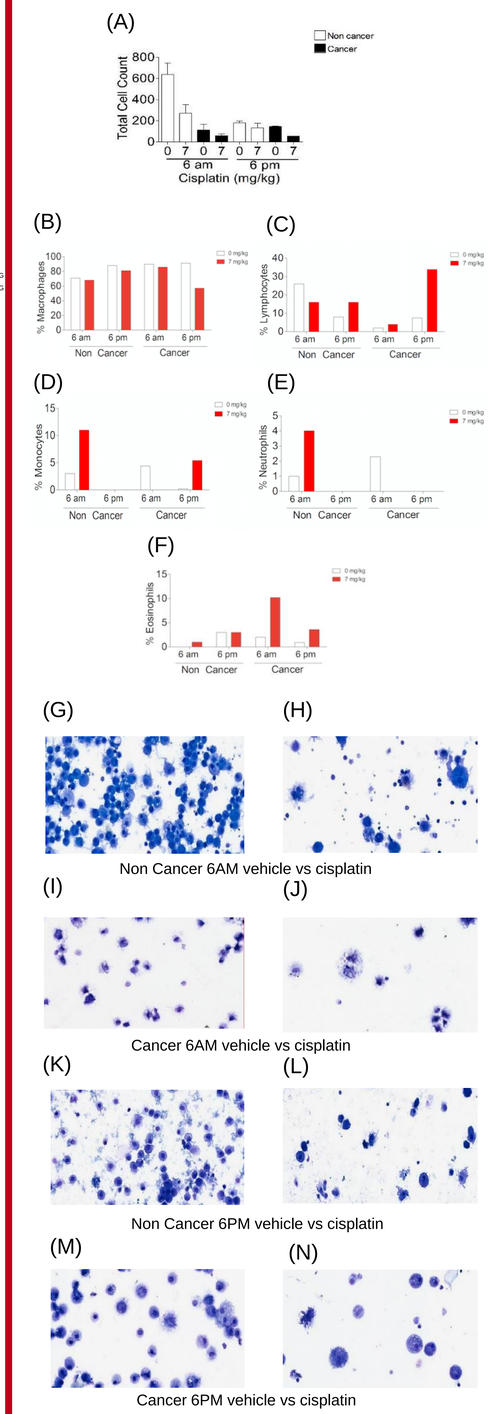



**Figure 13. Immune cells in the lungs via bronchoalveolar lavage fluid.** 40 wk old cancerous and noncancerous mice were treated with 7 mg/kg cisplatin or saline once a week for 4 weeks at either 6AM or 6PM and sacrificed at Day 24. (A) Total Immune Cell Counts were quantified and (B)-(F) Different immune cell populations were examined. (G)-(N) Representative images.

## Conclusions

- *Bmal1* shows most variation with cisplatin treatment at 6am versus 6pm in both cancerous and noncancerous mice.
- Circadian rhythm markers *Clock, Per2, Cry2,* and *Rev-erbα* were altered with CDDP treatment, indicating CDDP dysregulates circadian function in the kidney.
- Overall changes in kidney injury (tubular damage and fibrosis) at the different time points examined were not drastically different.
- In non cancer mice, OCT2 is depressed with cisplatin at both 6am and 6pm, but not at noon. This may indicate circadian dysregulation and/or difference in proximal tubule cell injury.
- **Understanding how circadian mechanisms are altered may play an important role in how patients are ultimately treated with CDDP.**

## Acknowledgments


Support for these studies was provided by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grant R01- DK093462, to L.J.S], NCI R25 grant support University of Louisville Cancer Education Program NIH/NCI (R25-CA134283), and the University of Louisville Undergraduate Research Scholar Award.