Repeated administration of cisplatin increases EGFR/EGFR activation and renal fibrosis in Kras4bG12D lung adenocarcinoma-bearing mice, but kidney injury is further exacerbated with erlotinib/cisplatin combination treatment

Kennedy M. Walls,[1] Cierra N. Sharp,[1] Mark A. Doll,[1] Tess V. Dupre,[1] Levi J. Beverly[2] and Leah J. Siskind[1]

[1] Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY

[2] Department of Medicine, University of Louisville, Louisville, KY

Abstract

Background:

Cisplatin (CDDP) is a first-choice therapy for many cancers, but 30% of patients develop acute kidney injury (AKI), which can progress to chronic kidney disease (CKD). Currently, there are no therapeutic interventions for CDDP-induced AKI or CKD. Clinically, only cancer patients receive CDDP, and it is administered in repeated, low doses to curtail CDDP nephrotoxicity. We optimized a repeated dosing regimen of CDDP (7 mg/kg 1x/wk for 4wks), which causes CKD in mice.

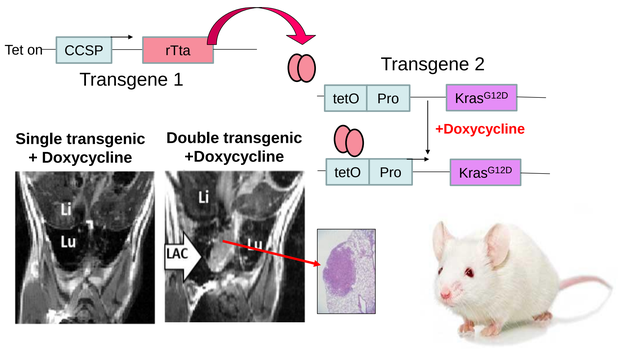
Methods:

To incorporate cancer into our model, we utilized a Kras4bG12D transgenic mouse that develops lung adenocarcinoma, and treated non-cancer and cancer mice with repeated CDDP dosing. Indices of kidney injury, function, and renal fibrosis were obtained using ELISAs, QRTPCR, and IHC staining for repeated CDDP studies and subsequent erlotinib studies.

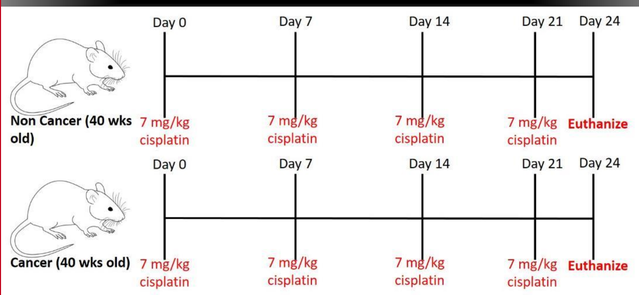
Results:

CDDP-treated cancer mice had lower survival (50%), and worsened fibrosis as indicated by Sirius red (SR) staining (25.4% SR +) and levels of myofibroblasts (α-SMA IHC; 4.6% +) compared to CDDP treated non cancer mice (11.6% SR +, 2.2% α-SMA +). Western blot analysis indicated that cancer mice treated with CDDP had increased EGFR and pEGFR Y1068 levels. Thus, we hypothesized that treating cancer mice with erlotinib (an EGFR inhibitor) in combination with CDDP would decrease EGFR activation and thereby decrease renal fibrosis. Administration of erlotinib as a renoprotective strategy (25 mg/kg once a day for 7 days) with a single dose of 7 mg/kg CDDP exacerbated renal damage and loss of function (NGAL: 1.30x10^6 pg/ml; BUN: 120 mg/dl) compared to the CDDP only group (NGAL: 4.99x105 pg/ml; BUN: 38.6 mg/dl). Additionally, we used erlotinib as an injury-ameliorating agent by administering erlotinib (25 mg/kg once a day for 30 days) after repeated dosing of CDDP in cancer mice. More data is needed to determine if intervening with erlotinib at this time point affects the development of renal fibrosis.

Kras4bG12D Transgenic Model of Lung Adenocarcinoma



Repeated Administration of Cisplatin



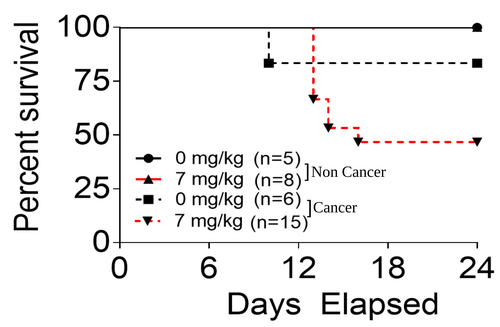


Figure 1. Repeated administration of cisplatin decreases survival of cancer-bearing mice. 40 wk old non cancer and cancer mice (Kras4bG12D transgenic model) were treated with 7 mg/kg cisplatin once a week for 4 weeks and sacrificed at Day 24.

Acknowledgements

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Results

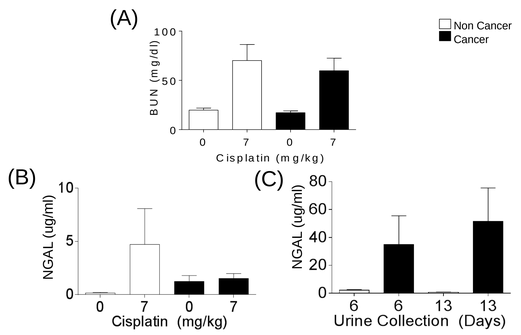


Figure 2. Markers of kidney function and injury. 40 wk old non cancer and cancer mice (Kras4bG12D transgenic model) were treated with 7 mg/kg cisplatin once a week for 4 weeks and sacrificed at Day 24. (A) Blood urea nitrogen (BUN) was measured in plasma. (B) Neutrophil gelatinase-associated lipocalin (NGAL) was measured in urine at day 24. (C) NGAL measured in urine at days 6 and 13 in cisplatin treated mice.

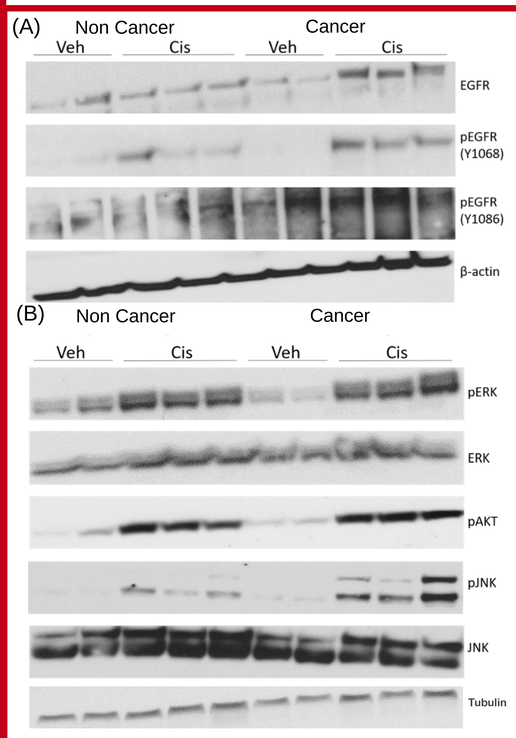


Figure 3. EGFR and EGFR-mediated signaling pathways. 40 wk old non cancer and cancer mice (Kras4bG12D transgenic model) were treated with 7 mg/kg cisplatin once a week for 4 weeks and sacrificed at Day 24. Western blots were done on kidney cortex homogenates to determine protein levels of (A) EGFR, pEGFR (Y1068), and pEGFR (Y1086), as well as (B) pERK, ERK, pAKT, pJNK, and JNK.

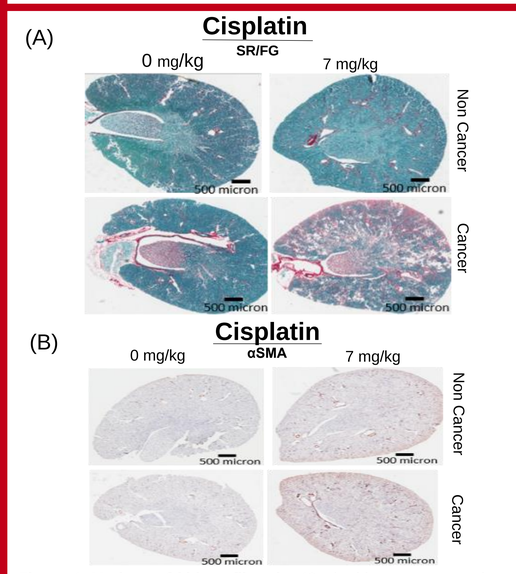
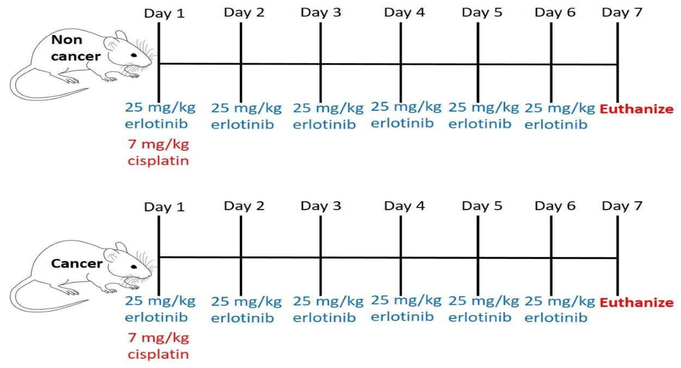


Figure 4. Markers of kidney fibrosis. 40 wk old non cancer and cancer mice (Kras4bG12D transgenic model) were treated with 7 mg/kg cisplatin once a week for 4 weeks and sacrificed at Day 24. (A) Total collagen deposition was determined by Sirius red/ Fast green staining. (B) Presence of myofibroblasts were determined via IHC staining for α-SMA.

Co-treatment with Cisplatin + Erlotinib



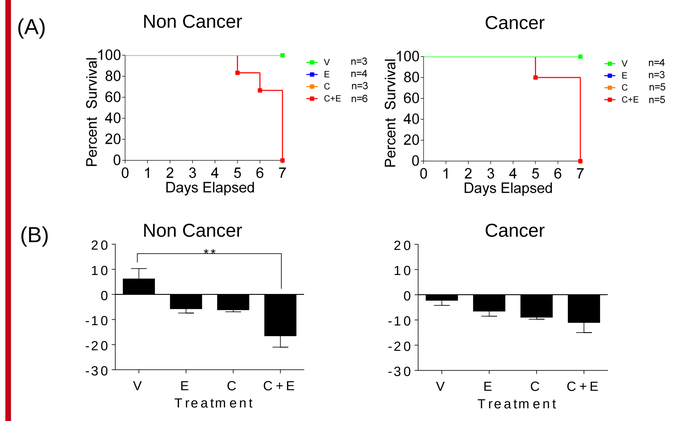


Figure 5. Survival and weight loss with cisplatin + erlotinib. 40 wk old non cancer and cancer mice (Kras4bG12D transgenic model) were treated with vehicle (V), erlotinib (E), cisplatin (C), or cisplatin and erlotinib (C+E). (A) Percent survival to Day 7. (B) Total weight loss. Statistical significance was determined by one-way ANOVA; ** indicates p<0.01.

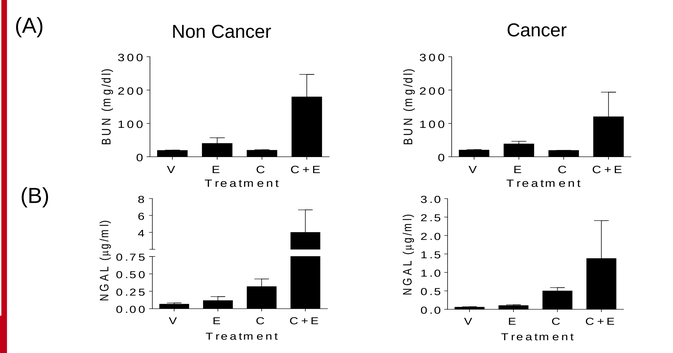
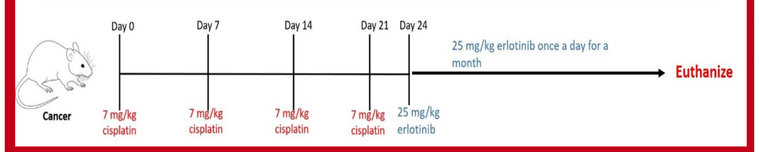


Figure 6. Markers of kidney function and injury. 40 wk old non cancer and cancer mice (Kras4bG12D transgenic model) were treated with either vehicle (V), erlotinib (E), cisplatin (C), or cisplatin and erlotinib (C+E). (A) Blood urea nitrogen (BUN) was measured in plasma. (B) Neutrophil gelatinase-associated lipocalin (NGAL) was measured in urine.

Treatment with Erlotinib After Cisplatin



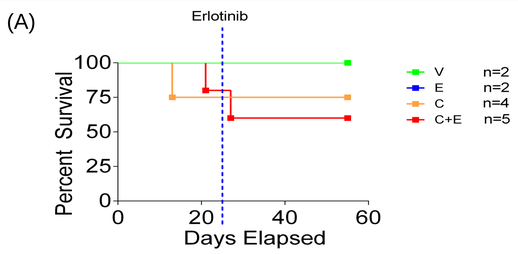


Figure 7. Survival with cisplatin + erlotinib. 40 wk old cancer mice (Kras4bG12D transgenic model) were treated with vehicle (V), erlotinib (E), cisplatin (C), or cisplatin followed by erlotinib (C+E). (A) Percent survival to Day 54.

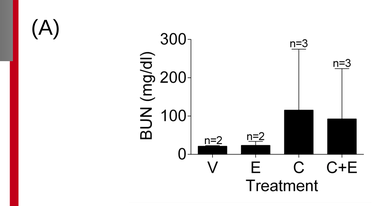


Figure 8. Markers of kidney function and injury. 40 wk old cancer mice (Kras4bG12D transgenic model) were treated with either vehicle (V), erlotinib (E), cisplatin (C), or cisplatin followed by erlotinib (C+E). (A) Blood urea nitrogen (BUN) was measured in plasma.

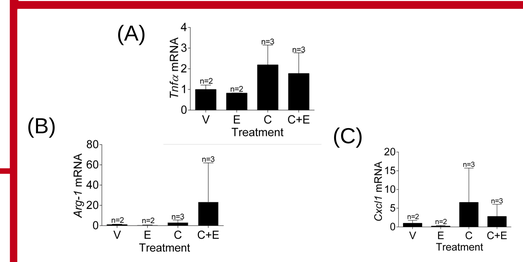


Figure 9. Inflammatory cytokines and chemokines with cisplatin + erlotinib. 40 wk old cancer mice (Kras4bG12D transgenic model) were treated with vehicle (V), erlotinib (E), cisplatin (C), or cisplatin followed by erlotinib (C+E). Inflammatory cytokines (A)*Tnfa* and (B) *Arg-1* and inflammatory chemokine (C) *Cxcl-1* were measured in kidney cortex via QRTPCR.

Figure 10. Markers of kidney fibrosis. 40 wk old cancer mice (Kras4bG12D transgenic model) were treated with 7 mg/kg cisplatin once a week for 4 weeks, followed by 25 mg/kg erlotinib once a day for 30 days and sacrificed at Day 54. (A) Total collagen deposition was determined by Sirius red/ Fast green staining. (B) Presence of myofibroblasts were determined via IHC staining for α-SMA.

Conclusions

- Repeated administration of low dose cisplatin in Kras4bG12D lung adenocarcinoma-bearing mice leads to:
  - a significant decrease in overall survival compared to age-matched non-cancer mice.
  - earlier and more severe peaks in NGAL levels
  - worsened renal fibrosis
- There are higher levels of EGFR/ pEGFR in cancer mice treated with cisplatin.
- These data suggest that targeting EGFR or EGFR-mediated pathways may be protective against cisplatin-induced kidney injury in cancer patients specifically.
- Combination of cisplatin and erlotinib produced severe AKI, preventing age-out studies of long-term effects.
- Intervention with erlotinib at a later time point did not reduce or reverse the presence of fibrosis.

Erlotinib is not a good therapeutic choice as a renoprotective agent in cisplatin-induced kidney injury, but more data is needed to determine if erlotinib is a good therapeutic choice as an injury-ameliorating agent in cisplatin-induced kidney injury.

Future Studies

- Determine which cell type(s) have upregulated EGFR in the kidney
- Use erlotinib at varying time points during cisplatin dosing schedule to see if fibrosis can be reversed when intervening at different times during cisplatin treatment
- Utilize more samples in the “Treatment with Erlotinib After Cisplatin” experiment and then age out the mice to determine long-term effects of erlotinib intervention