Inhibition of Autophagy Protects from Cisplatin-Induced Kidney Injury in a Repeated Low Dose Model

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INTRODUCTION

Cisplatin (CDDP) is a common chemotherapy agent used to treat solid-organ tumors. 30% of patients given CDDP develop acute kidney injury and the other 70% are at risk for chronic kidney disease. Acute and chronic CDDP-induced kidney injury can be modeled in rodents, leading to different pathological outcomes. While autophagy has been shown to protect from CDDP-induced AKI, its role in CDDP-induced kidney fibrosis is unknown.

Autophagy is a cellular recycling process that destroys and reconstructs damaged cells. Previously, our lab has observed decreased levels of kidney functional loss and fibrosis in mice that receive a repeated low dose CDDP treatment and Chloroquine, a late-stage autophagy inhibitor. To ensure the effects of Chloroquine could confidently be attributed to inhibition of autophagy, we inhibited an earlier stage of autophagy with 3-methyladenine (3MA) and found similar effects.

HYPOTHESIS

We hypothesize that autophagy plays a harmful role in CDDP-induced kidney injury and fibrosis.

METHODS

Vehicle	3-MA	Cisplatin	Cisplatin + 3-MA
Saline 1x/week	15 mg/kg 3-MA 3x/week	9 mg/kg CDDP 1x/week	9 mg/kg CDDP 1x/week + 15 mg/kg 3-MA 3x/week

Figure 1. Dosing schedule, throughout 3 weeks.

- Mice were treated via IP injection according to the dosing schedule above.
- Markers of renal function, cell injury, inflammation, and fibrosis were assessed post-euthanasia.
- Whole kidneys were homogenized for flow cytometric analysis and 1 million events were recorded per sample.
- Data was analyzed using ANOVA one-way and expressed as ± SEM.
- Criterion of statistical difference was p<0.05 for all comparisons.

RESULTS

Kidney Injury and Functional Loss was Prevented by Autophagy Inhibition

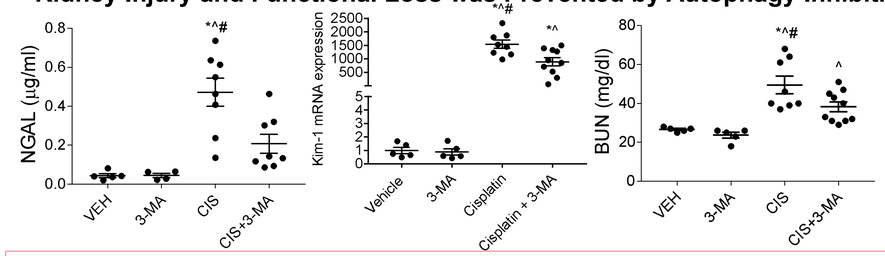

Figure 2. NGAL measured in urine, Kim-1 mRNA measured in kidney cortex, BUN measured in serum.

Autophagy Inhibition Does Not Block Cytokine and Chemokine Expression

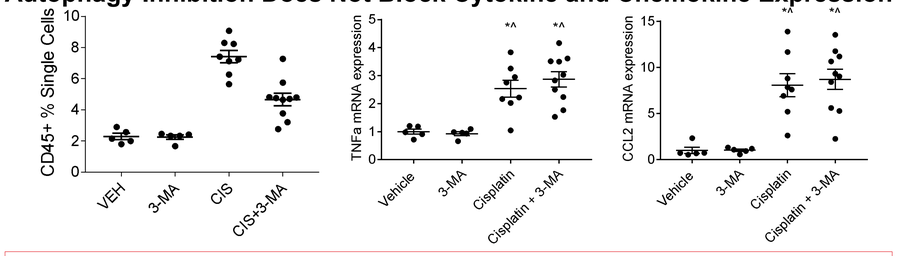

Figure 3. CD45 measured by flow cytometry, TNFα and CCL2 mRNA measured in kidney cortex.

Development of Renal Fibrosis was Decreased by Autophagy Inhibition

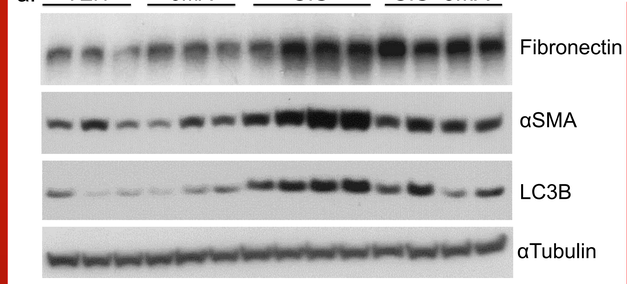

Figure 4. 3-MA prevents CDDP-induced fibrotic development.
4a (left). Western blot of fibrotic (fibronectin, αSMA) and autophagy (LC3B) markers.
4b (below). Timp-1, ICAM-1, Col 1a1 mRNA measured in kidney cortex.

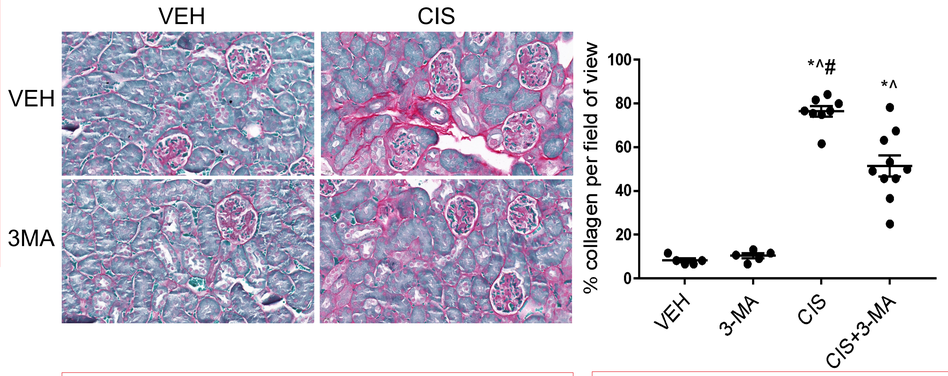

Figure 5. 3-MA reduces collagen deposition in CDDP-induced kidney disease. Sirius Red/Fast Green stain (SR/FG), 40x image.

Figure 6. Visual quantitation of SR/FG stain.

Key
*: p<0.05 from Vehicle; ^: p<0.05 from 3-MA; #: p<0.05 from Cis
VEH: Control with saline injection, 3-MA: 3-methyladenine, CIS: Cisplatin

AUTOPHAGY PATHWAY

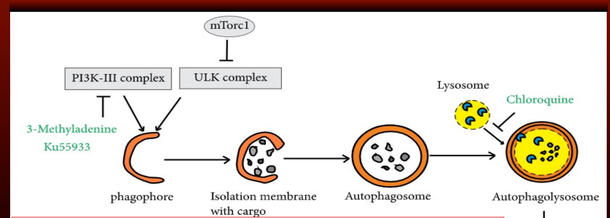

Figure 7. 3-MA inhibition in the autophagy pathway
Adapted from: https://www.hindawi.com/journals/bmri/2019/4087160/

CONCLUSIONS

- The inhibition of autophagy by 3-MA has:
  - Attenuated kidney injury and functional loss.
  - Blocked infiltration of total immune cells but did not block renal production of inflammatory cytokines and chemokines.
  - Reduced the progression of renal fibrosis.

ACKNOWLEDGEMENTS

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FUTURE STUDIES

- Investigate cell type specificity of the protective effects of autophagy inhibition.
- Investigate the role of recruiting cytokines and chemokines and infiltrating immune cells in the protective effects of autophagy inhibition.