Inhibition of PARP-1 Attenuates Rat Renal Ischemia Reperfusion Injury

Christina Bracken[1], Effie Tozzo[1], Katelyn Pulito[1], Jeff Stanwix[1], Robert W Shine[1], Mahati Krishna[2], Nan Ji[1], Dominique Stickens[1]
[1]Mitobridge, Inc. Cambridge, MA 02138; [2]Syngene International, Bangalore, India

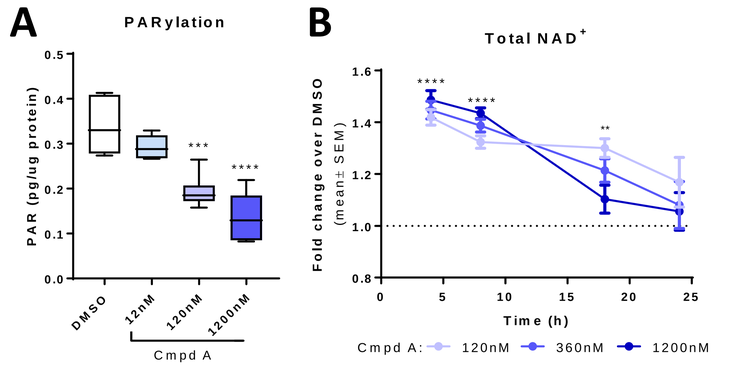
Background

Acute renal ischemia reperfusion injury (IRI) generates superoxide and other reactive species that activate PARP-1 to repair ROS-mediated DNA strand breaks. However, PARP-1 activation depletes the cells from NAD⁺ and ATP, and promotes pro-inflammatory signaling, exacerbating kidney injury. We hypothesized that PARP-1 inhibition would decrease/prevent NAD⁺ depletion and reduce ischemia-reperfusion-induced acute kidney injury (IR-AKI), offering a potential therapeutic treatment for AKI.

Methods

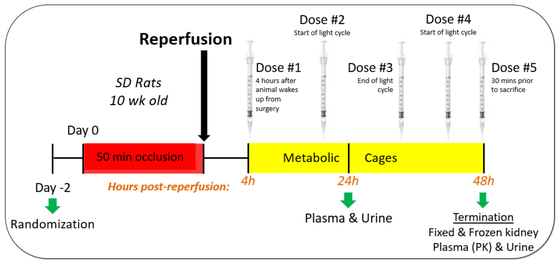
In vitro: We evaluated the ability of Cmpd A, a novel and selective PARP-1 inhibitor, to boost NAD⁺ and mitochondrial respiration in control and cisplatin challenged human renal proximal tubule epithelial cells (PTCs). In vivo: Sprague-Dawley rats underwent a 50 minute bilateral IR and were administered Cmpd A (or vehicle), twice daily at 0.1, 0.3, 1, 5, or 15 mg/kg by intravenous injection for 2 days, beginning at 4 hours post reperfusion. Cmpd A activity was assessed by measuring renal PAR levels, NAD⁺ and its breakdown products, by ELISA or mass spectrometry. Kidney injury biomarkers were measured in plasma at 24 and 48hr post reperfusion and tubular injury was evaluated by histopathology. Gene expression analysis for inflammatory mediators and *Vegfa* were assessed using Nanostring technology. Statistical analysis was performed using GraphPad Prism. Sham vs. IRI compared using Student's T-test. #p<0.05, ###p<0.001, ####p<0.0001. Cmpd A treated cells or animals were compared to DMSO or IRI using One-Way ANOVA followed by post hoc Dunnett's test. *p<0.05, **p<0.01,***p<0.001 and ****p<0.0001

Cmpd A Boosts NAD⁺ and Mitochondrial Respiration, Reduces PAR, and Rescues Cisplatin-Induced NAD⁺ Depletion in Human PTCs



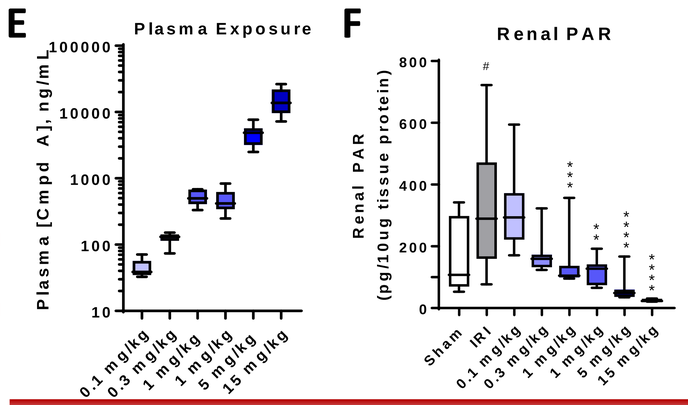
PARP-1 inhibitor, Cmpd A reduces PAR levels (**A**), and boosts NAD⁺ (**B**) in human PTCs translating to increased basal and maximal respiration (**C**). hTERT RPTECs are treated with Cmpd A for 24 hours then analyzed from total NAD⁺ and PAR by ELISA, and oxygen consumption using Seahorse. Importantly, in HK2 cells challenged with a sub-lethal dose of cisplatin, which reduces NAD⁺, Cmpd A is able to fully rescue the CDDP-mediated NAD⁺ loss (**D**).

IR-Induced AKI in Rats



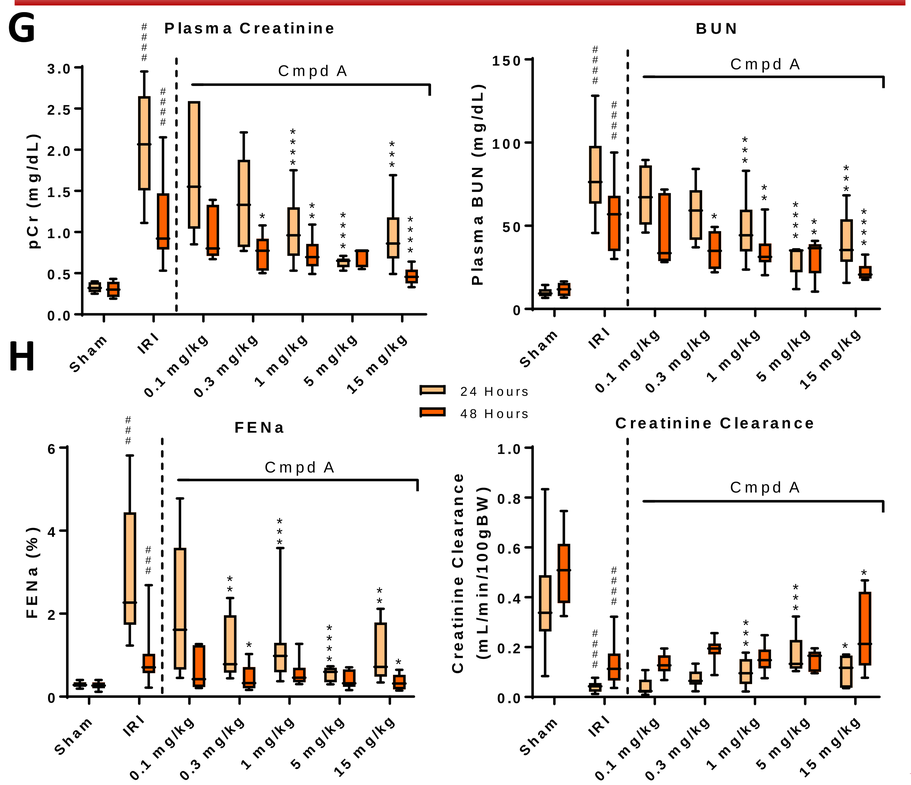
We evaluated the ability of Cmpd A, a novel and selective PARP1 inhibitor, to mitigate IR-induced AKI in rats. Cmpd A was IV injected at 0.1, 0.3, 1, 5 or 10 mg/kg beginning 4 hours post reperfusion. Plasma & urine were collected at 24h and 48h post reperfusion to assess AKI biomarkers, termination was at 48 hours to assess compound activity, NAD⁺ catabolism, gene expression and histopathology.

Cmpd A *in vivo* Activity



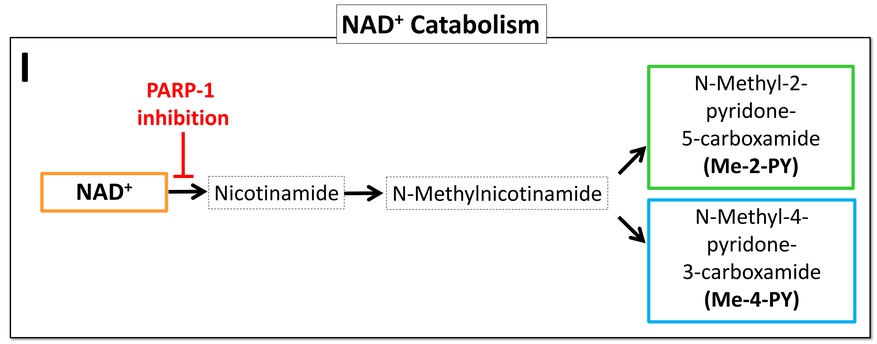
Cmpd A is dose-proportionally increased in rat plasma 30 mins post-dose. Similar results (not shown here), were obtained in kidney tissue (**E**).
PAR levels measured in the kidney cortex from Cmpd A treated rats are inversely correlated to exposure (**F**).

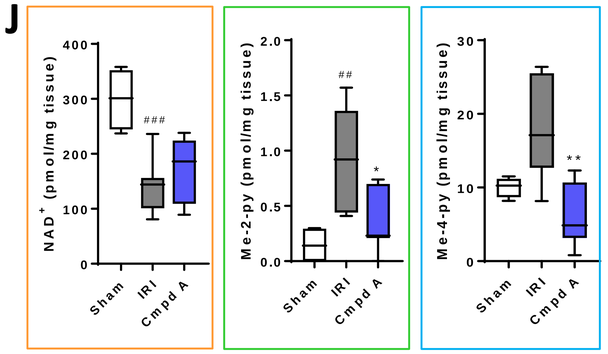
PARP-1 Inhibition Attenuates IR-AKI *in vivo*



At 24 hours, (after just two IV doses), Cmpd A dose-dependently reduces plasma biomarkers of acute kidney injury (**G**), translating to a restoration of renal function (**H**). As injury resolves at 48 hours, Cmpd A sustains reduction of AKI biomarkers and maintains renal function.

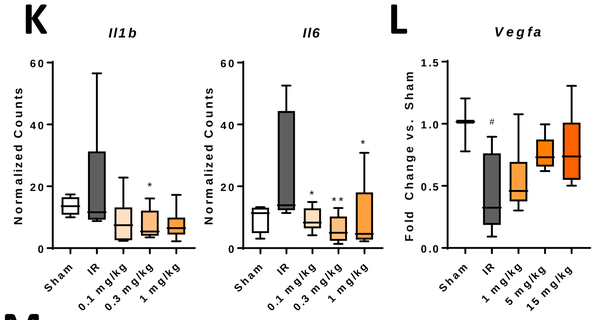
Cmpd A Blocks NAD⁺ Catabolism In Vivo





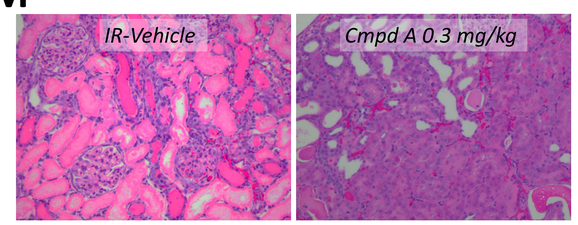
IR-AKI leads to an enhanced NAD⁺ consumption and an increase of its downstream metabolites Me-2-Py and Me-4-py (**I**). NAD⁺ and these breakdown products were measured by Mass-Spec in renal cortex samples. Cmpd A blocks NAD⁺ catabolism leading to an increase of NAD⁺ concomitant with increased Me-2-py and Me-4-py (**J**).

PARP-1 Inhibition Reduces Inflammation and Mitigates Vascular Dropout



PARP-1 directly interacts with and activates the NF-κB-mediated response to inflammatory stimuli[1]. Consistent with PARP-1 KO mice showing reduced kidney expression of pro-inflammatory cytokines following IR-AKI[2], Cmpd A downregulates *Il1b* and *Il6* gene expression in our study (**K**). Additionally, Cmpd A dose-dependently reduces the loss of *Vegfa* gene expression (**L**), suggesting a reduced vascular dropout following IR-AKI[3].

Finally, mediated in part by reduced inflammation and vascular dropout, Cmpd A partially restores the normal tubular architecture shown by H&E staining (**M**).



Acknowledgements and References

The authors thank Dr. Bharat Lagu for providing Cmpd A and the Mitobridge and Astellas teams for helpful discussions. Please refer to FR-PO078 for a description of the IR-AKI model.

References: [1]El-Hamoly et al., Mol. Med. 2014.; [2]Zheng et al., Am J Physiolo Renal Physiol 2005.; [3]Basile et al., Am J Physiol Renal Physiol. 2008.