Methods:

8-12 weeks old Male Sprague Dawley (SD) rats (Harlan, Indianapolis, IN) were maintained with a standard chow food and allowed free access to water. The protocol was approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. Cisplatin (Sigma Chemical, St. Louis, MO) was dissolved in normal saline to make a fresh working solution of 1.5mg/ml. SD rats were administered a single oral dose of 2.5 mg/kg body weight of Bardoxolone Methyl (BARD) or vehicle on day -1 (n=10 for each treatment). On day 0, BARD and vehicle treated SD rats received a dose of 6 mg/kg body weight cisplatin (n=6 for each group) or normal saline (n=4 for each group) through tail vein injection. The animals were sacrificed for analysis 5 days after cisplatin administration. In another set of the experiments, a single dose of 10 mg/kg of BARD or vehicle was administered, as schematized in Figure 1A.

Blood serum samples were collected every other day for blood urea nitrogen (BUN) and creatinine levels. Kidney tissues were collected, and subjected to hematoxylin and eosin (H&E) staining. Renal structural damage was scored using a semi-quantitative scoring system based on the percentage of damaged tubules as previously described (1): 0 (no damage), 1 (mild or <25%), 2 (moderate, 25~50%), 3 (moderate-severe, 50~75%), and 4 (severe or >75%). Western blot analysis was performed for heme oxygenase-1 (HO-1) expression as described (2). All data are presented as the mean ± SEM. Statistical analysis was performed by using t-test for comparisons involving two groups and defined as significant at $P < 0.05$.

Results:

A single dose of cisplatin at 6mg/kg body weight resulted in significantly elevated serum BUN and creatinine levels at day 3 and 5. Treatment with 2.5mg/kg BARD resulted in a modest decrease in BUN and creatinine levels (Supplemental Figure 1B and C), however treatment with 10 mg/kg BARD significantly attenuated the rise in BUN and serum creatinine levels following cisplatin administration ($P<0.01$) (Supplemental Figure 1D and E). BARD or vehicle alone had no adverse side effects on renal function at any time points.

Cisplatin-induced renal structural damage was markedly reduced in renal cortical and outer medullary regions in the BARD treated (10mg/kg body weight) animals (Supplemental Figure 1F-J). The expression of HO-1, a key downstream target of the Keap-1-Nrf2 pathway, was increased three fold in the BARD- compared to vehicle-treated animals (Supplemental Figure 1K and I).

References:

1) Molitoris BA, Dagher PC, Sandoval RM, Campos SB, Ashush H, Fridman E, Brafman A, Faerman A, Atkinson SJ, Thompson JD, Kalinski H, Skaliter R, Erlich S, and Feinstein E. siRNA targeted to p53 attenuates ischemic and cisplatin-induced acute kidney injury. J Am Soc Nephrol 20: 1754-1764, 2009.

2) Chen B, Guo L, Fan C, Bolisetty S, Joseph R, Wright MM, Agarwal A, and George JF. Carbon monoxide rescues heme oxygenase-1-deficient mice from arterial thrombosis in allogeneic aortic transplantation. Am J Pathol 175: 422-429, 2009.

Figure legend

Figure 1: BARD treatment attenuates cisplatin-induced renal injury.

A: Outline of the experimental protocol. Male SD rats were given a single dose of 2.5 mg/kg or 10mg/kg body weight of BARD or vehicle (n=10/group) on day -1. Both groups received 6 mg/kg body weight cisplatin (n=6) or normal saline (n=4) by intravenous injection on day 0. Serum was collected at 1 day before, and 1, 3, and 5 days after cisplatin administration. Animals were sacrificed for analysis on day 5. **B-E: Effect of BARD treatment on renal function.** Serum creatinine and BUN levels in BARD and vehicle-treated animals using 2.5mg/kg body weight **(B and C)** or 10 mg/kg body weight **(D and E)** of BARD. *P<0.02, **P<0.01 between BARD and vehicle-treated animals. **F-J: Histological evaluation.** H&E staining of renal cortex **(F and G)** and outer medulla **(H and I)** in vehicle (**F and H**) and 10 mg/kg BARD (**G and I**) treated animals. **(J)** Semi-quantitative analysis of renal histology in BARD and vehicle-treated animals and scored from 0-4 as described in the methods. (**K)** Western blot showing induction of HO-1 protein in the kidney from the animals with BARD or vehicle-treatment 5 days after cisplatin administration. (**L)** Densitometric analysis of the western blot with relative intensity of HO-1 expression to the internal control actin.