Modulation of PPARδ with MTB-2 post-reperfusion attenuates IR-induced AKI injury biomarkers and histopathology in rats

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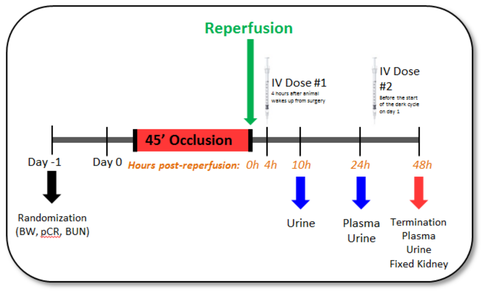
Background

Ischemic acute kidney injury (AKI) is characterized by persistent proximal tubule mitochondrial dysfunction. Due to their highly oxidative metabolism, proximal tubule cells utilize fatty acids to generate the energy required for their specialized function. We hypothesized that enhancing fatty acid oxidation with a potent and highly selective PPARd modulator will restore mitochondrial function, offering a potential therapeutic treatment for AKI.

Methods and Analysis

Animal model: Sprague-Dawley (SD) rats underwent 45 minutes of bilateral renal pedicle ischemia followed by reperfusion. Post-reperfusion, MTB-2 was dosed IV QD for 2 days; termination was at 48 hours. Two separate studies were preformed to test doses from 0.3 to 10 mg/kg of MTB-2 IV. **Plasma biomarkers and renal function:** At indicated time points, urine volume was measured, creatinine and Na were analyzed in urine and plasma, BUN was analyzed in plasma using a clinical analyzer. GFR (creatinine clearance) and FENa were calculated according to standard calculations. Cystatin C was analyzed in plasma by ELISA at 24 and 48 hr post-reperfusion. **Urinary biomarkers:** Timp-2 and IGFBP-7 concentrations were determined by ELISA in urine collected from 4-10h post reperfusion; the resultant concentrations were multiplied and divided by 1000 to generate a rat Nephrocheck® equivalent value. Urinary FABP-1 and NGAL were analyzed by ELISA at 24 and 48 hr post-reperfusion, respectively. Data was normalized to the urine creatinine concentration of the same sample. **Histology:** Formalin-fixed, paraffin embedded kidney tissues were sectioned and stained with H&E, scoring was done by a Board Certified veterinary pathologist.

Figure 1: Study Design



Statistics: Depending on the result of a normality test, Sham-vehicle vs IR-vehicle significance was tested an unpaired t-test (normal) or Mann-Whitney test (not normal). Treatment groups were compared to IRI-vehicle either by ANOVA with Dunnett's post-hoc (normal distribution) or Kruskal-Wallis with Dunn's post-hoc (not normally distributed). In all statistical analysis, a *p*-value of less than 0.05 was taken to be statistically significant.

#p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 Sham-Vehicle vs. IR-Vehicle, *p<0.05 **p<0.01, ***p<0.001, ****p<0.0001 IR-Vehicle vs. MTB-2. n=4 Sham-Vehicle, n=5-6 IR-Vehicle, n=7 0.3 mg/kg, n=7 1 mg/kg, n=8 3 mg/kg, n=8 5 mg/kg, n=7 10 mg/kg.

MTB-2 reduces clinically relevant plasma biomarkers

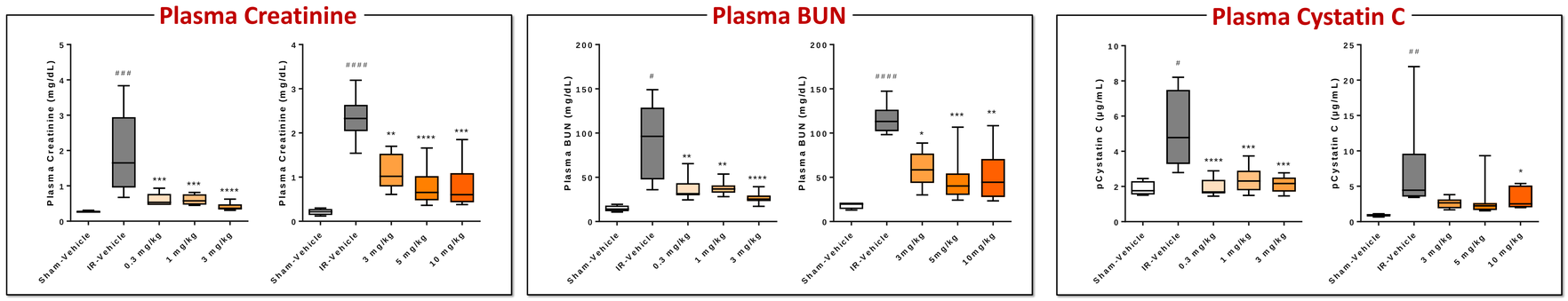


Figure 3: Modulation of PPARδ reduces clinically relevant plasma biomarkers. All doses of MTB-2 from 0.3 through 10 mg/kg tested reduced plasma creatinine, BUN and cystatin C at 24 hours post dose. Similar data was obtained at 48 hours post dose.

MTB-2 reduces urinary biomarkers of AKI

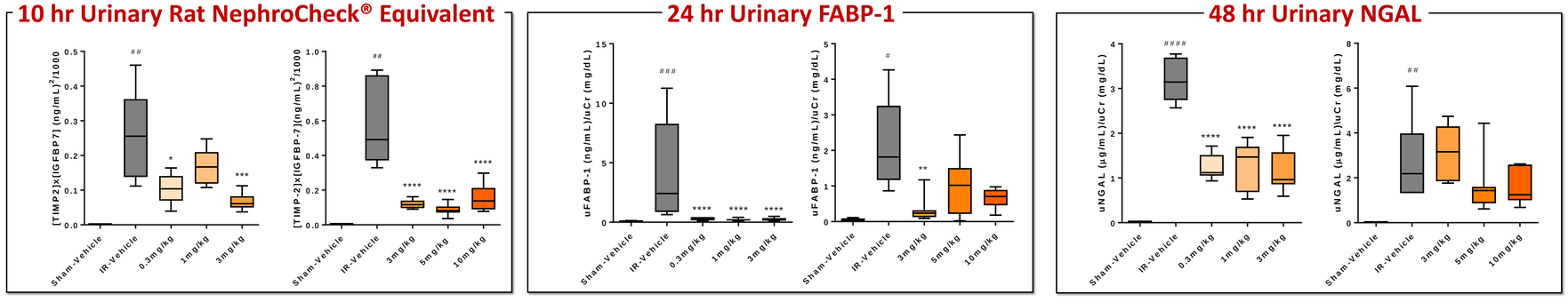


Figure 4: Modulation of PPARδ reduces urinary biomarkers. In urine collected continuously from 4 through 10 hours post reperfusion, the NephroCheck® equivalent value was significantly reduced with MTB-2 treatment. At 24 and 48 hours post reperfusion, MTB-reduces urinary FABP-1 and NGAL, respectively.

MTB-2 restores renal and tubular function

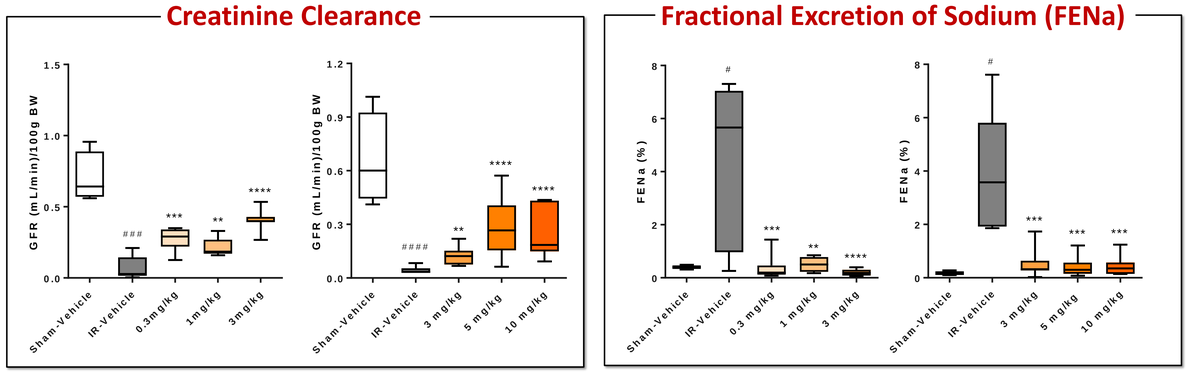


Figure 2: Modulation of PPARδ restores renal and tubular function during AKI. MTB-2 attenuates IR-induced loss GFR, as well as reduces IR-induced FENa. Data presented is at 24 hours post reperfusion, similar data was obtained at 48 hours.

MTB-2 reduces tubular injury

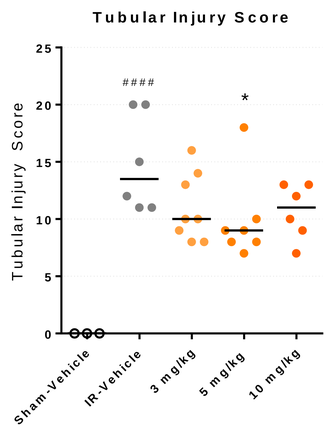


Figure 5: Tubular injury is mitigated by PPARδ modulation post reperfusion. At 48 hours post injury there is significant tubular injury. The score at left represents the sum of tubular necrosis, dilation, presence of tubular casts and loss of brush border. MTB-2 reduces the overall tubular injury score.

Conclusions and future directions

- Our data demonstrates that selective PPARd modulation after an ischemic AKI event in rats is sufficient to recover renal and tubular function, reduce clinically relevant urinary and plasma injury biomarkers and improve kidney histopathology.
- In preclinical safety studies MTB-2 was shown to be safe and well tolerated. The next step will be to test this therapeutic in clinical trials. Developing clinical plan in partnership with Astellas Pharma

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