MA-0204 modulation of PPARδ protects and promotes recovery after AKI in normal rats and aged diabetic CKD Zsf1 rats by enhancing fatty acid oxidation in proximal tubular epithelial cells.

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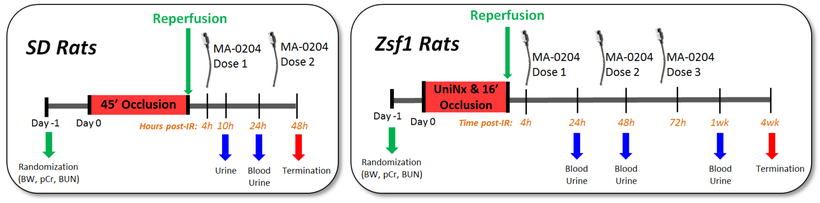
Background

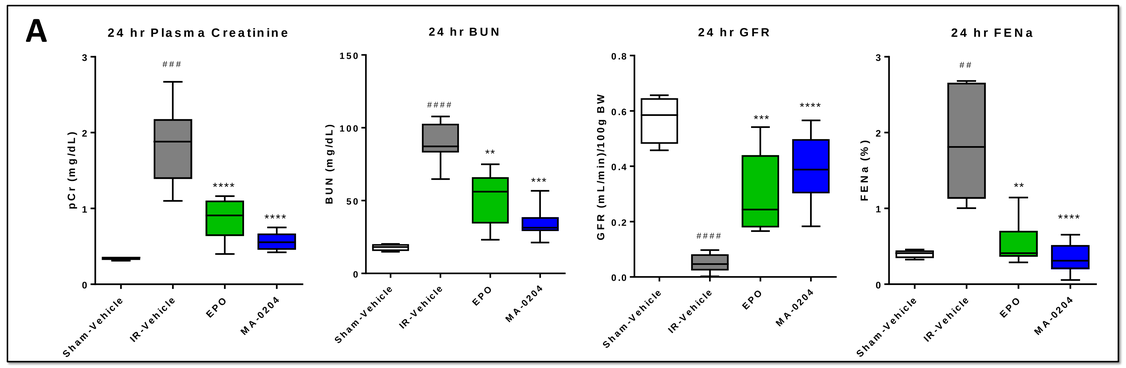
Ischemic acute kidney injury (AKI) is characterized by persistent proximal tubule mitochondrial dysfunction. Due to their highly oxidative metabolism, proximal tubule cells utilize fatty acids to generate the energy required for their specialized function. We hypothesized that enhancing fatty acid oxidation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

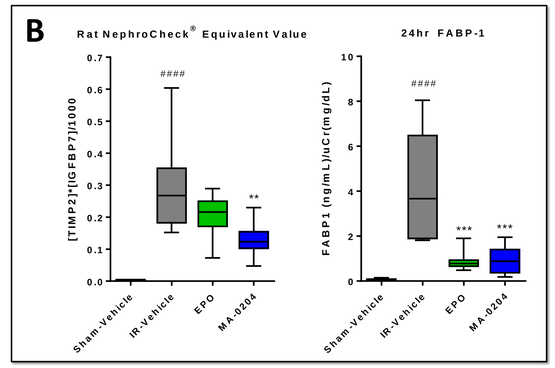
Methods

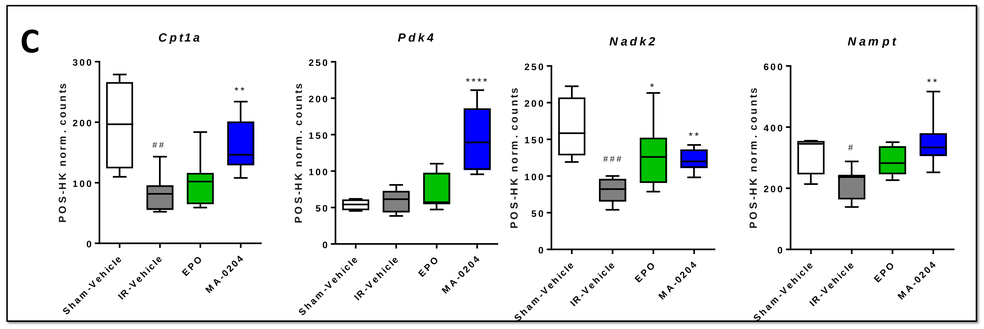
In vitro assays: Human hTERT RPTECs were treated with 70nM MA-0204 for 24 hr, then analyzed for gene expression by qPCR and their ability to utilize palmitate to drive mitochondrial oxygen consumption by Seahorse. **Animal models:** Sprague-Dawley (SD) rats underwent 45 minutes of bilateral renal pedicle ischemia followed by reperfusion. Post-reperfusion, MA-0204 or vehicle were dosed orally, QD for 2 days; termination was at 48 hours. Aged (18 wk-old), obese, diabetic, CKD Zsf1 rats underwent 16 minutes of ischemia and removal of one kidney. Following reperfusion MA-0204 or vehicle were dosed QD for 3 days; termination was 4 weeks post AKI. For both experiments, EPO served as a positive control and was dosed IV at 1000 U/kg 30 mins prior to ischemia. **Clinical chemistry:** At indicated time points, urine volume was measured, creatinine and Na were analyzed in urine and plasma, BUN and glucose were analyzed in plasma using a clinical analyzer. GFR and FENa were calculated according to standard calculations. **Urinary biomarkers:** Timp-2 (LSBio) and IGFBP-7 (MyBioSource) concentrations were determined in urine collected from 4-10h post reperfusion, the resulting concentrations were multiplied and then divided by 1000 to generate a rat Nephrocheck® equivalent value. Urinary FABP1 (R&D Systems) was measured in 24 hr samples and normalized to 24 hr urinary creatinine values. Urinary TNFR (Aviva) was assessed at baseline and 4 weeks in Zsf1 rats, values are reported relative to baseline. **Kidney cortex gene expression** analysis was conducted using Nanostring. **Cumulative Histopathology** scores were generated by the sum of tubular necrosis, dilation, presence of tubular casts and loss of brush border. Individual scoring was done by a Board Certified veterinary pathologist. **Hydroxyproline** content in 4 wk-old Zsf1 kidneys was assessed using an assay kit (Sigma) according to manufacturer's instructions.

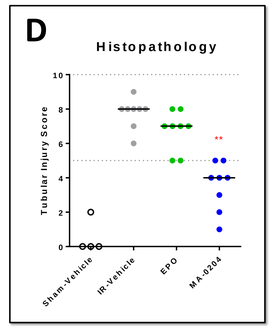


PPARδ Modulation Recovers Renal Function and Reduces Tubular Injury in SD Rats





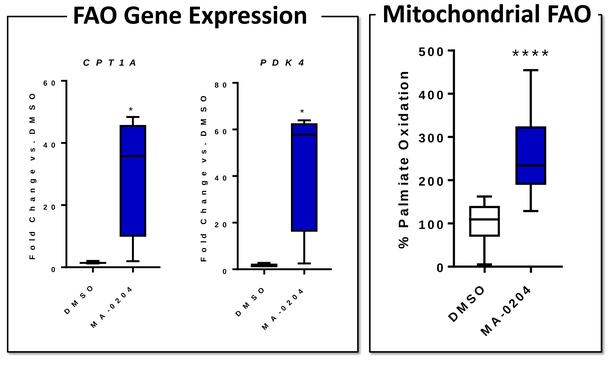




#p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 Student's unpaired t-test vs. IR-Vehicle; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, One-Way ANOVA vs. IR-Vehicle followed by Dunnett's test; n=4 Sham-Vehicle, n=8 IR-Vehicle , n=7 IR-Vehicle (*Nadk2*, FABP-1) or n=6 IR-Vehicle (FENa), n=8 EPO, n=8 MA-0204

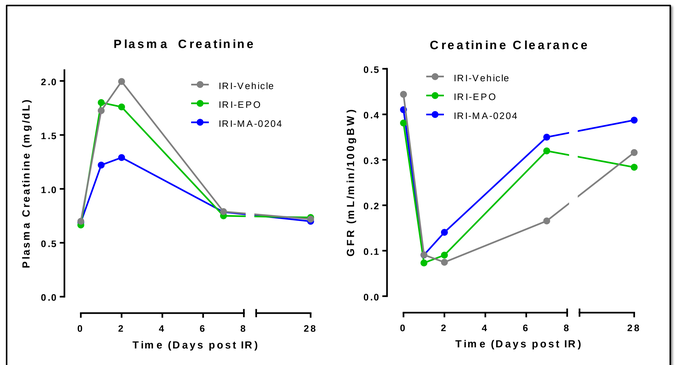
(**A**) At 24 hours post reperfusion, AKI plasma biomarkers (Creatinine, BUN) and functional readouts (GFR, FENa) were attenuated with MA-0204. (**B**) MA-0204 also reduced the NephroCheck® score, and urinary FABP-1. Note that 24 hour data represents the results of a single dose, at 48 hours similar results were obtained (results of 2 doses, data not shown). (**C**) PPARδ target genes *Cpt1a* and *Pdk4* were upregulated in kidney cortex of MA-0204 treated rats suggesting activation of fatty acid oxidation. IR-AKI induced a deficit of NAD^+ metabolism genes *Nadk2* and *Nampt,* this was restored by MA-0204. (**D**) MA-0204 mitigated the significant tubular damage that occurred in IR-AKI animals 48 hours post injury.

MA-0204 Enhances Fatty Acid Oxidation (FAO)



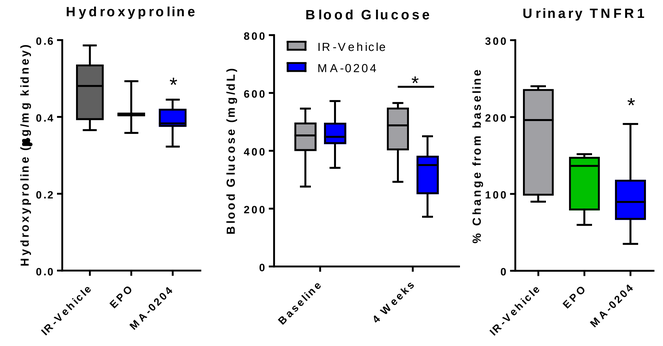
MA-0204 activates PPARδ transcriptional targets *PDK4* and *CPT1A*, translating in a functional response of RPTECs to increase oxidation of fatty acid palmitate. Cells were treated with MA-0204 for 24 hours then assayed for gene expression, or fed palmitate and assayed for oxygen consumption rate *p<0.05, ****p<0.0001, Students' unpaired t-test (gene expression), or Mann-Whitney test (Seahorse as), n=18 DMSO, n=17 MA-0204; two independent experiments.

PPARδ Modulation Attenuates AKI and its Long Term Sequelae in Zsf1 Rats



Modulation of PPARδ by MA-0204 attenuates IR-AKI-induced increase of plasma creatinine at 24 and 48 hr post surgery. Functionally, this translates to a faster recovery of renal function measured by MA-0204-mediated increased creatinine clearance compared to IR-Vehicle, beginning 2 days post injury and sustained throughout the 4 weeks. N=5-10 IR-Vehicle, n=4-6 EPO, n=10-12 MA-0204. Group median values are presented.

MA-0204 acute dosing in an AKI-CKD model reduces co-morbidities 4 wks later. Zsf1 rats received 3 doses of MA-0204 after IR-AKI + UniNx. Four wks later, treated rats had reduced hydroxyproline content in cortical tissue, suggesting less fibrotic tissue. MA-0204 resulted in better diabetes control at 4 wks, indicated by reduced blood glucose. MA-0204 attenuated urinary TNFR1 excretion at 4 wks, suggesting PPARδ modulation slowed progression of CKD.



*p<0.05, One way ANOVA vs. IR-Vehicle followed by Dunnett's test (Hydroxyproline, TNFR1) or Student's unpaired t-test (Glucose) vs IR-Vehicle. n=6 IR-Vehicle (4 wks) or n=10 IR-Vehicle (baseline), n=3-4 EPO, n=9 MA-0204

Conclusions

1) MA-0204 is a highly potent and selective PPARδ modulator that upregulates the expression of FAO genes in human renal proximal tubule cells, resulting in increased mitochondrial fatty acid oxidation.
2) In normal rats undergoing IR-AKI, MA-0204 attenuates the increases of plasma and urinary biomarkers of AKI, improves renal function, mitigates proximal tubular damage and boosts mitochondrial gene expression.
3) In severely obese, diabetic, CKD, aged Zsf1 rats undergoing IR-AKI and uni-nephrectomy, MA-0204 attenuates the acute increase in plasma creatinine, achieves faster and sustained recovery of renal function and reduces markers of co-morbidities.

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