Human Recombinant α_1-Microglobulin Protects Against Acute Kidney Injury in Rat Models of Ischemia-Reperfusion Injury (IRI)

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ABSTRACT

BACKGROUND

Acute kidney injury (AKI) is a global health concern associated with high morbidity, mortality, and progressive chronic kidney disease (CKD). RMC-035, a recombinant human α_1-microglobulin (A1M), has demonstrated protective antioxidant effects in several injury models. The principal mechanisms of RMC-035 are heme binding, reductase activity, radical scavenging and protection of mitochondria. RMC-035 is being developed for the prevention of cardiac surgery associated-AKI, and are currently evaluated in a Phase 1 study. We present preclinical data for RMC-035, supporting its protective effect in ischemic AKI and AKI on a CKD background.

METHODS

RMC-035 (0-5 mg/kg, i.v.) was administered at various time-points and doses prior and/or post renal ischemia in rats exposed to unilateral nephrectomy followed by a 30 minute pedicle clamp ischemia. AKI was evaluated at 1-5 days post injury by serum creatinine (sCr), BUN and 24 hr urinary creatinine clearances (CrCl). Furthermore, RMC-035 (2 mg/kg, i.v.) was administered prior to and post renal clamp ischemia in rats previously subjected to unilateral and renal ischemia ("AKI on CKD model"). Texas Red-x labeled RMC-035 (TR-x-RMC-035) trafficking and handling by proximal tubule cells (PTC) was studied via intravital imaging.

RESULTS

RMC-035 caused a dose-dependent decrease in AKI measured as reduced proteinuria, sCr and BUN levels, and improved 24 hr CrCl, in rats subjected to a single renal IRI episode. RMC-035 given prior and post renal IRI was more effective for protection vs. a single dose given either before or after IRI. In a CKD model with two successive episodes of AKI over 28 days, RMC-035 given at the second IRI episode significantly reduced renal injury by sCr and CrCl. TR-x-RMC-035 was rapidly filtered and bound to the apical brush border in PTC. Accumulation of RMC-035, tubular-vesicular extensions and vesicular trafficking was seen from 30 minutes through 24 hr post infusion. Cytosolic release was seen as early as 70 minutes.

CONCLUSION

RMC-035 demonstrates dose-dependent protective effects against AKI in multiple IRI models including AKI on CKD, had a prolonged PTC half-life including release into the cytosol, thus being a novel and promising therapeutic candidate for the treatment of cardiac surgery associated-AKI.

METHODS

ANIMALS

Male and female Sprague-Dawley or Munich Wistar Frömter rats in fed condition were used for all studies at 8-12 weeks of age. Inactin was used as an anesthetic for all short term studies. Isofluorane was used when recovery and re-imaging was conducted. All procedures were approved by IACUC.

AKI- and AKI on CKD-MODEL

Sprague-Dawley rats were anesthetized using isofluorane, 5% induction 1-2% maintenance at 1L/min 02. For the AKI model the pedicle of the left kidney was cleaned and the renal vein and artery were clamped for 40 minutes with a simultaneous right kidney nephrectomy. The CKD model followed the AKI model protocol with an additional 30 minutes of ischemia to the remaining left kidney followed by utilization 4 weeks after the 2nd renal ischemic event.

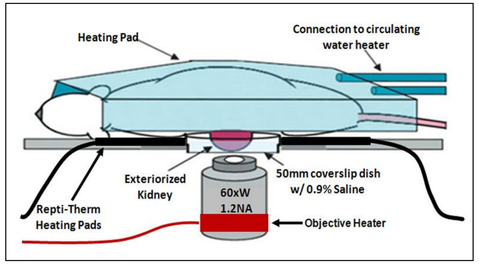
AKI was evaluated at 1-7 days post injury by sCr, BUN and 24 hr CrCl.

RMC-035 TREATMENT

Treatment with RMC-035 (0-5 mg/kg) or vehicle, administered as an i.v. dose just prior to clamping followed by an additional dose either at 15 minutes or 4 hr post-reperfusion.

INTRAVITAL 2-PHOTON MICROSCOPY

Intravital imaging to assess trafficking and renal handling of TR-x-RMC-035 was conducted as previously described and shown below. To determine cytosolic release, single plane images were background subtracted and a 12-bit binary mask highlighting the lysosomes was generated.



STATISTICS

Values are presented as individual data and box plots displaying medians and 25th and 75th percentiles. Differences between RMC-035 treated animals vs. Vehicle were analyzed using the Mann-Whitney U-test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

RESULTS

RMC-035 reduces sCr and BUN in AKI-model

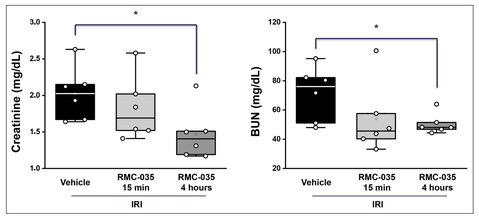


Figure 1. Analysis of sCr and BUN, at 24 hr following unilateral nephrectomy and 30 minutes unilateral renal pedicle clamping in rats. RMC-035 treated rats, receiving one dose of 5 mg/kg prior to the clamping in combination with either one dose 15 minutes post-reperfusion (light gray, n=6) or 4 hr post-reperfusion (grey, n=6). Vehicle treated animals are displayed in black (n=6).

RMC-035 improves 24 hr CrCl and reduces proteinuria in AKI-model

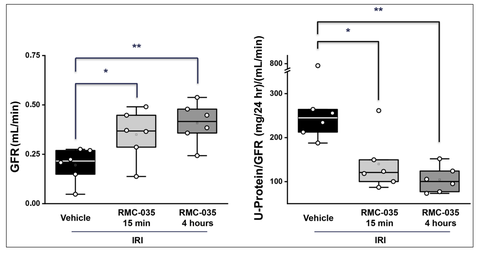


Figure 2. Analysis of GFR and proteinuria, at 72 hours following unilateral nephrectomy and 30 minutes unilateral renal pedicle clamping in rats. RMC-035 treated rats, receiving one dose of 5 mg/kg prior to the clamping in combination with either one dose 15 minutes post-reperfusion (light gray, n=6) or 4 hours post-reperfusion (grey, n=6). Vehicle treated animals are displayed in black (n=6).

CONCLUSION

RMC-035 demonstrates dose-dependent protective effects against AKI in multiple IRI models including AKI on CKD, had a prolonged PTC half-life including release into the cytosol, thus being a novel and promising therapeutic candidate for the treatment of cardiac surgery associated-AKI

RESULTS

RMC-035 dose-dependent reduction in sCr and BUN in AKI-model

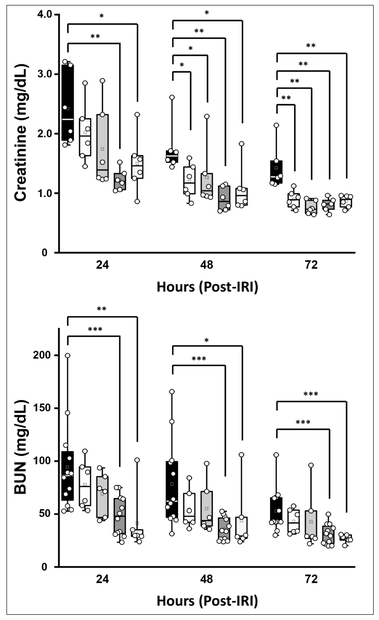


Figure 3. Analysis of sCr and BUN, at 24-72 hours following unilateral nephrectomy and 30 minutes unilateral renal pedicle clamping in rats. RMC-035 (0-5 mg/kg) treated rats, receiving one dose prior to clamping and a 2nd dose 4 hr post-reperfusion. Vehicle in black (sCr n=6, BUN n=12), 0.5 mg/kg RMC-035 in white (n=6), 1.0 mg/kg in light grey (n=6), 2.0 mg/kg in grey (sCr n=6, BUN n=12) and 5.0 mg/kg in white dotted (n=6).

RMC-035 reduces sCr in AKI on CKD-model

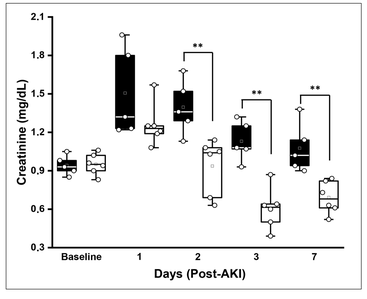


Figure 4. Analysis of sCr at baseline, days 1, 2, 3 and 7 in male/female CKD rats following 30 minutes of unilateral renal pedicle clamping. RMC-035 treated rats (2 mg/kg), receiving one dose prior to the clamping and one dose 4 hr post-reperfusion. Treated animals are displayed as follows: Vehicle in black (n=5) and 2.0 mg/kg RMC-035 in white (n=6).

RESULTS

RMC-035 improves 24 hr CrCl in AKI on CKD-model

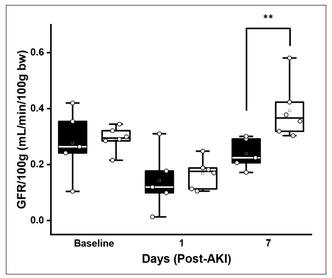


Figure 5. Analysis of GFR (mL/min/100 g bw) at baseline, days 1 and 7 in male/female CKD rats following 30 minutes of unilateral renal pedicle clamping. RMC-035 treated rats (2 mg/kg), receiving one dose prior to the clamping and one dose 4 hr post-reperfusion. Treated animals are displayed as follows: Vehicle in black (n=5) and 2.0 mg/kg RMC-035 in white (n=6).

PTC uptake and cytosolic release of TR-x-RMC-035

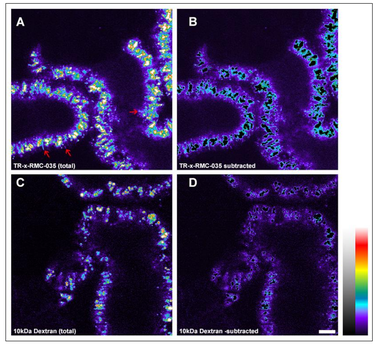


Figure 6. Renal handling of TR-x-RMC-035 evaluated by intravital imaging. Panel A shows TR-x-RMC-035 uptake in an S1 segment ~ 70 minutes post infusion. Panel B shows the result of subtracting lysosomal fluorescence. Note the hazy appearance in cytosol of the tubular epithelia and trafficking extensions from the endocytic pool (red arrows). In contrast, images from a dextran (Panel C) processed identically (Panel D) show little cytosolic haze. Bar=10µm. Pseudo-color scale shown next to Panel D.

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