DEVELOPMENT OF AN "*IN SITU*" RENAL PERFUSION SYSTEM TO STUDY THE ORIGIN OF URINARY BIOMARKERS IN TWO ANIMAL MODELS OF ACUTE KIDNEY INJURY INDUCED BY GENTAMICIN AND ISCHEMIA-REPERFUSION

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Introduction

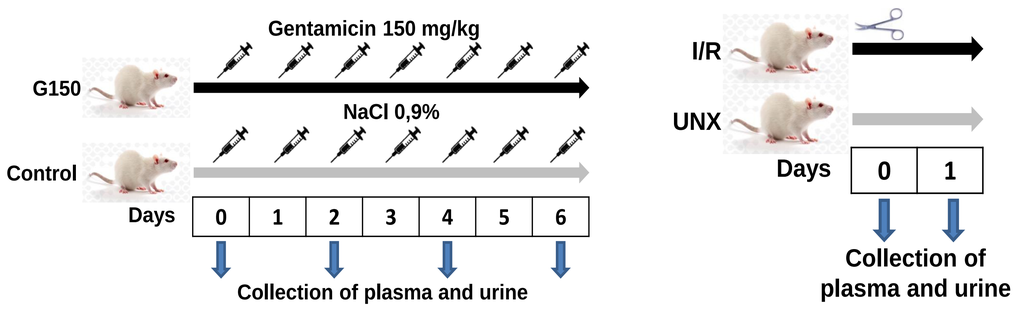
Gentamicin is an aminoglycoside antibiotic widely used for the treatment of many infectious diseases. Its main side effect is nephrotoxicity, which occurs in 10-25% of therapeutic courses, despite proper monitoring and hydration of patients, can lead to acute kidney injury (AKI). Moreover ischemia-reperfusion (I/R) injury is a major cause of AKI in native kidneys and in renal allograft, and it is associated with a high rate of mortality in patients and enhanced rate of rejections in transplanted kidneys. We have previously demonstrated that urinary damage markers like kidney injury molecule 1 (KIM-1) and regenerating islet-derived protein III beta (REGIIIβ) increased in AKI animal models.

Aims

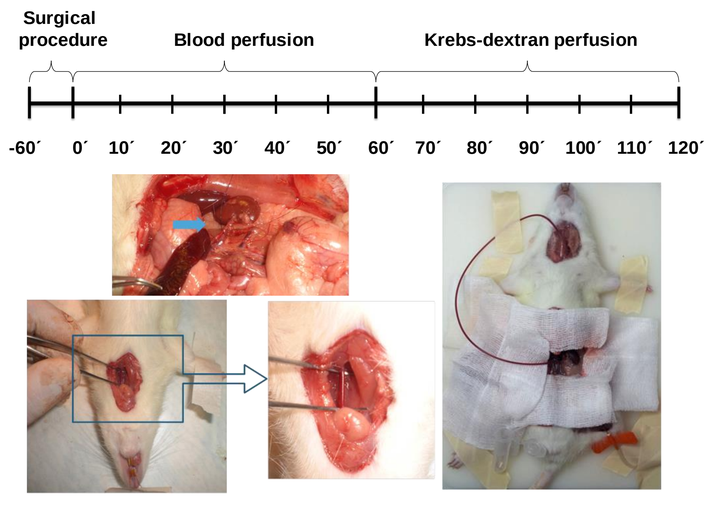
In the present work we aimed at specifically studying the renal handling of these markers in two AKI animal models, through in situ renal perfusion experiments.

Methods / experimental design

Male Wistar rats were administrated by a single dose of gentamicin (150 mg/kg) or not, and animals *underwent* one hour of warm renal ischemia or not.



At the time of maximum renal damage, rats were anesthetized and an extracorporeal circuit for kidney perfusion was set up. The renal artery, vein and ureter of the right kidney were ligated. The renal artery of the left kidney and the urinary bladder were canulated. A catheter was placed in the right carotid artery and connected directly to the renal artery. Urine was continuously collected from a catheter placed in the urinary bladder at 10 minute intervals. After 1 hour of renal perfusion with blood from the carotid artery, oxygenated and warm (37 ºC) Krebs-dextran (40 g/L of dextran) was perfused through the renal artery at 3 mL/min, and was discarded through the renal vein. KIM1 and REGIIIb were measured in the different urine fractions.



Conclusions

KIM1 and REGIIIb are a low molecular weight protein freely filtered through the glomerular filtration barrier. Accordingly, our results using a complementary technique reinforce our previous studies, which indicated that the urinary KIM1 and REGIIIb observed in rats suffering of AKI result from the altered tubular handling of these proteins, most probably due to a defect in their tubular re-uptake.

Results

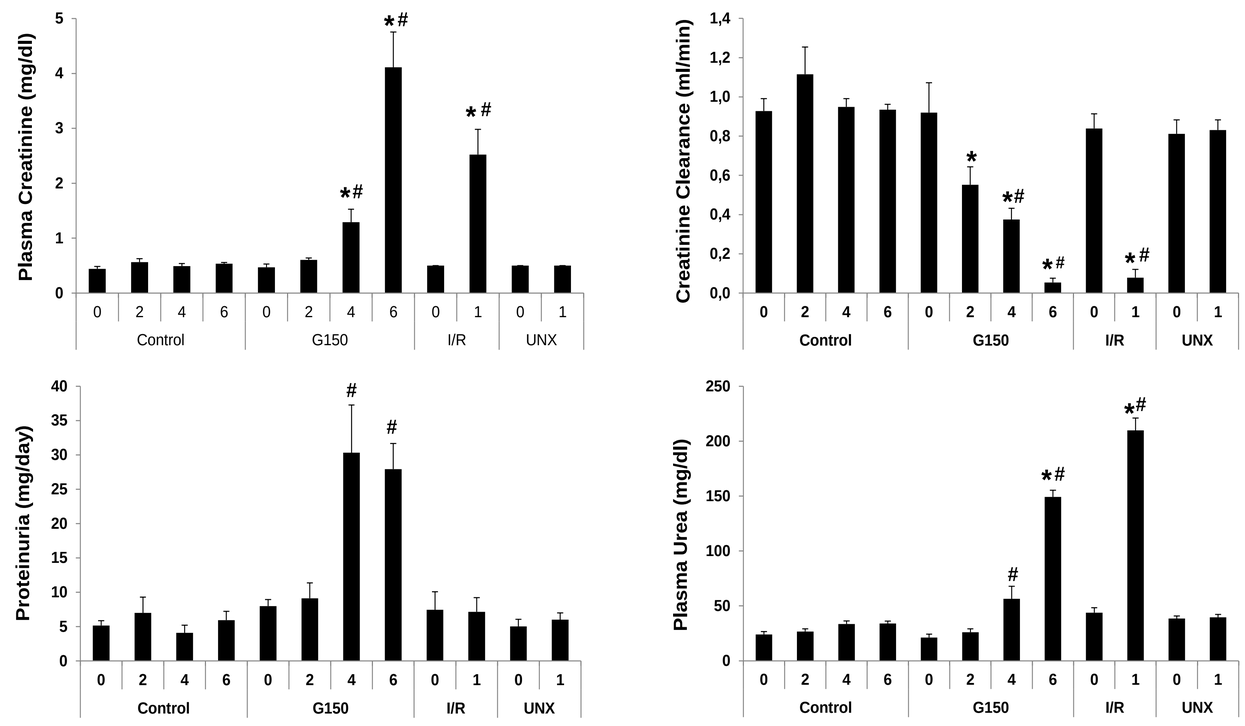


Fig 1. Plasma creatinine concentration, creatinine clearance, proteinuria and plasma urea concentration in control rats (Control; n=5), rats treated with gentamicin 150 mg/kg/day (G150; n=7)and in rats with 60 minutes of renal ischemia (I/R; n=4) or with a contralateral uninephrectomy (UNX; n=4). Statistically significant: *z>1,96 vs. control group (for G150) or vs. UNX group (for I/R) same day; #: z>1,96 vs. day 0 in the same group.

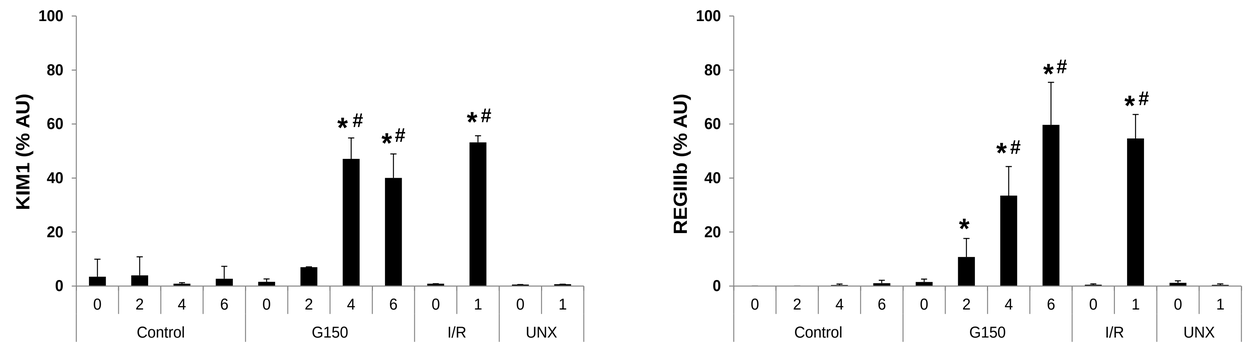


Fig 2. Evolution of urinary KIM1 and urinary REGIIIb excretion determined by western blot. Values are expressed as mean ± SEM (n=4). Statistically significant: *z>1,96 vs. control group (for G150) or vs. UNX group (for I/R) same day; #: z>1,96 vs. day 0 in the same group.

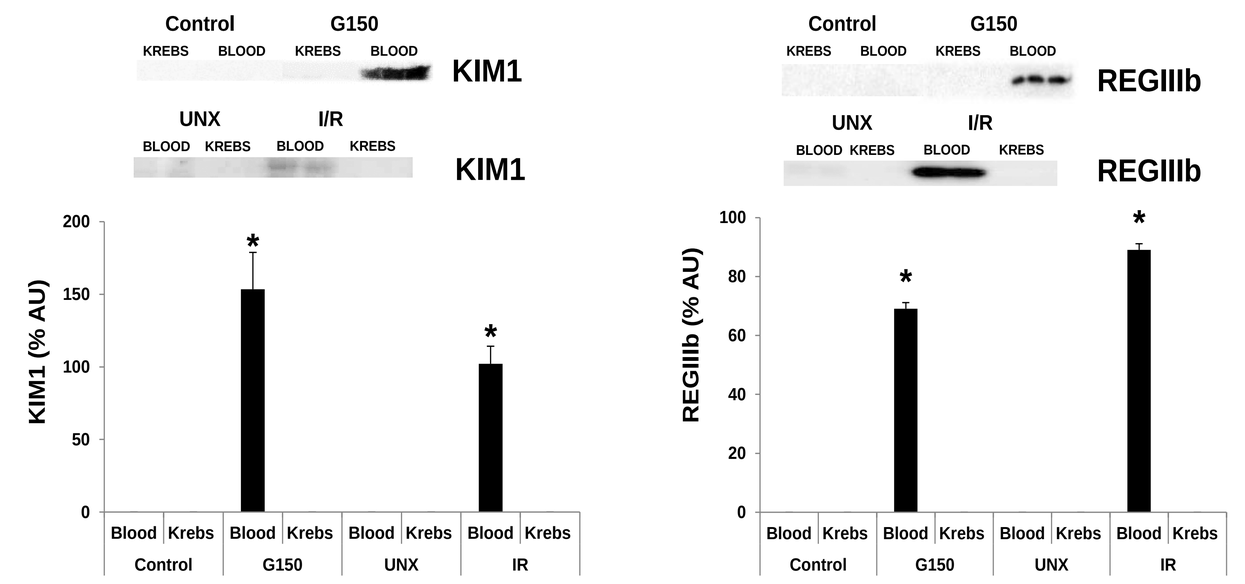


Fig 3. Excretion of urinary KIM1 and REGIIIb determined by western blot when the kidney is perfused with its own blood or with Krebs-dextran solution. Values are expressed as mean ± SEM (n=4). Statistically significant differences: *z>1,96 vs control group (for G150) or vs. UNX group (for I/R) same perfusion type.

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