Characterization of a bilateral ischemia-reperfusion-injury mouse model of acute kidney injury

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Background & Aim

Renal ischemia reperfusion injury (IRI) is one of the leading causes of acute kidney injury (AKI) which is an important risk factor for development of chronic kidney disease (CKD). Renal IRI involves temporary impairment and subsequent restoration of oxygen and nutrient delivery to kidney cells which determine long-term renal outcomes.

Here, we characterized the bilateral IRI (bIRI) mouse model of AKI and the nephroprotective effects of a standard NSAID (dexamethasone). Induction of bilateral temporary obstruction of renal blood flow induces albuminuria, increased plasma creatinine and urea and tubular injury. Treatment with dexamethasone improves the markers of nephropathy and reverse the regulation of several disease-associated genes.

Methods

See study outline in Fig. 1. Male C57BL/6J mice (9 weeks of age) were randomised into study groups based on body weight. On day 1, mice underwent bIRI surgery (32 min obstruction of blood flow of both kidneys) or sham surgery (n=10 per group). Sham and bIRI mice received vehicle or dexamethasone (5mg/kg, IP) once daily for 5 days starting three days before surgery. Mice were terminated two days after surgery. Terminal endpoints included urine biochemistry and histological evaluation (left kidney) of tubular injury and transcriptome signatures. Tubular injury is graded 0 to 6 based on the percentage of tubular necrosis and degeneration. Tubular dilation is scored from 0 to 2. Total histopathology is calculated based on the sum of all scorings.

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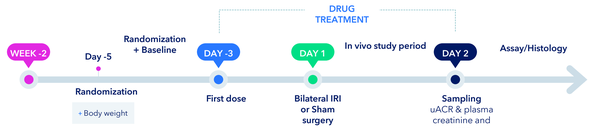
1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	Sham Vehicle	Male	10	**Vehicle**	IP	QD	5 ml/kg	-
2	bIRI Vehicle	Male	10	**Vehicle**	IP	QD	5 ml/kg	-
3	bIRI Dexamethasone	Male	10	**Dexamethasone**	IP	QD	5 ml/kg	**5 mg/kg**

Figure 1. Study outline. bIRI, bilateral ischemia reperfusion injury; IP, intraperitoneal.

2 Body and kidney weight

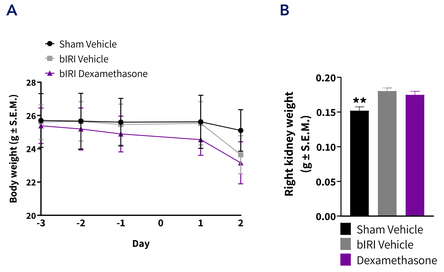


Figure 2. Body weight and kidney weights. (A) Body weight **(B)** Right kidney weight at termination (n = 10). **p<0.01 compared to Sham Vehicle (Dunnett's test one-factor linear model).

3 Biochemical markers of nephropathy

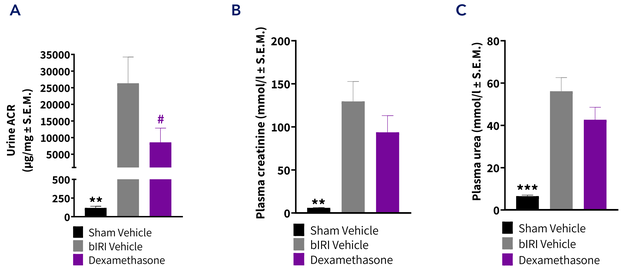


Figure 3. Dexamethasone improves biomarkers of nephropathy. (A) Albumin-to-creatinine ratio (ACR). **(B)** Plasma creatinine at termination. **(C)** Plasma urea at termination. n = 9-10., **p<0.01, ***p<0.001 compared to Sham Vehicle, #p<0.05 compared to bIRI Vehicle (Dunnett's test one-factor linear model).

4 Tubular necrosis

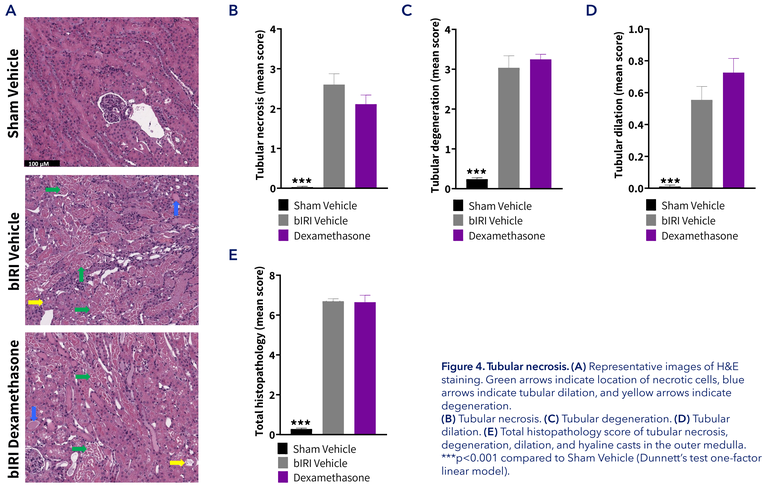


Figure 4. Tubular necrosis. (A) Representative images of H&E staining. Green arrows indicate location of necrotic cells, blue arrows indicate tubular dilation, and yellow arrows indicate degeneration. **(B)** Tubular necrosis. **(C)** Tubular degeneration. **(D)** Tubular dilation. **(E)** Total histopathology score of tubular necrosis, degeneration, dilation, and hyaline casts in the outer medulla. ***p<0.001 compared to Sham Vehicle (Dunnett's test one-factor linear model).

5 Transcriptomics profile

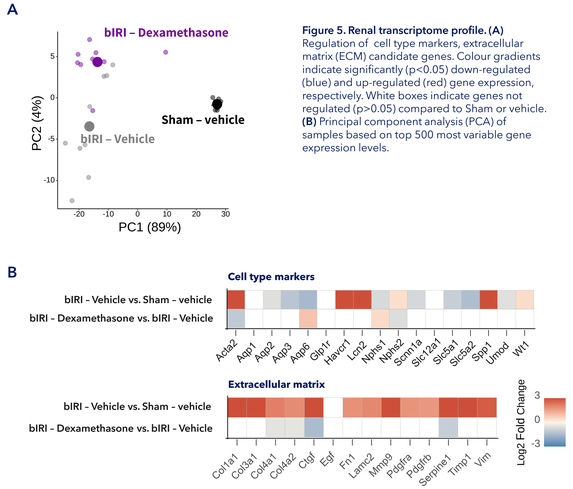


Figure 5. Renal transcriptome profile. (A) Regulation of cell type markers, extracellular matrix (ECM) candidate genes. Colour gradients indicate significantly (p<0.05) down-regulated (blue) and up-regulated (red) gene expression, respectively. White boxes indicate genes not regulated (p>0.05) compared to Sham or vehicle. **(B)** Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels.

Conclusion

- The bIRI mouse demonstrates significantly increases urine and plasma markers of nephropathy
- The bIRI mouse shows robust tubular necrosis, degeneration and dilation
- Dexamethasone improves biochemical and gene expression markers of nephropathy in the bIRI mouse

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