Establishing the Ischemia-Reperfusion Model in Rats for Chronic Renal Pathology Investigations

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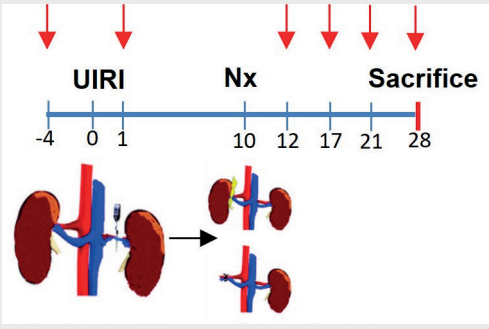
Introduction and Aim

Unilateral ischemia-reperfusion injury (UIRI) with contralateral nephrectomy (Nx) is a valuable model for acute and chronic renal injury research. Rats, due to their size and tolerance to the pathology, are the preferred lab animals for this model. However, while the model is well-established in mice [1], there are no established protocols for its implementation in rats [2]. Therefore, the aim of the study was to discover the peculiarities of kidney acute-to-chronic injury transition using UIRI-Nx model, depending on kidney occlusion time.

Methods

Young male SD rats were utilized in this study. UIRI was induced with occlusion times of 35, 40, and 45 minutes, followed by Nx on the 10th day post-UIRI. Animals were observed for up to 28 days post-UIRI (see Scheme 1). Serum creatinine and urea levels were monitored before the study initiation (blank levels), and on days 1, 12, 17, 21, and 28 post-UIRI. Kidney morphology was evaluated via Hematoxylin and Eosin staining in a semi-quantitative manner, kidney injury including the state of glomerular and tubular apparatuses, and kidney regeneration and inflammation (both interstitial and glomerular) were assessed as well. Renal fibrosis was assessed using Masson's trichrome staining by measuring the area occupied with collagen fibers, on day 28.



IRI by clamping left kidney vascular bundle for 35, 40, or 45 min

Right nephrectomy

Scheme 1. Study design. Red arrows indicate blood collection

Results

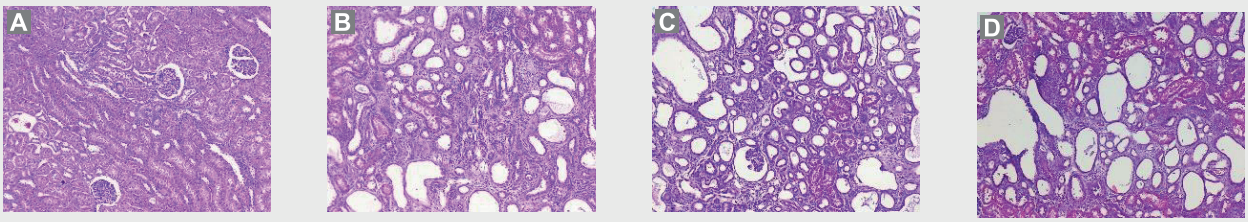


Figure 1. Microphotographs of injured kidney of rats 28 d after UIRI; H&E, Mg. x100. A – Sham, B – UIRI 35 min occlusion, C – UIRI 40 min occlusion, D - UIRI 45 min occlusion. Substantial tubular dilation, tubular epithelium flattening, desquamation, sometimes atrophy, and interstitial inflammation are observed in B-D.

Rats exhibited good tolerance to UIRI and subsequent Nx, with mortality rates not exceeding 20% regardless of occlusion time. Serum creatinine levels increased 1.5-2 times on day 1 post-UIRI, followed by a 5-6 fold increase post-Nx, gradually returning to baseline levels. Urea levels showed a more significant and prolonged increase, albeit returning to baseline by the study's end (Figure 2). While the observed changes showed consistency across all UIRI-Nx groups, they did not exhibit a strict correlation with occlusion time. Kidney injury severity (according to histopathological examination), as well as the expression of inflammation and regenerative capacity, were also slightly dependent on occlusion duration, demonstrating, however, substantial increase of the respective scores compared to Sham-operated animals (see Figures 1,2). At the same time, fibrosis development was notably influenced by occlusion duration, with longer occlusion periods resulting in more profound replacement of renal tissue with fibrotic one (see Figures 2,3).

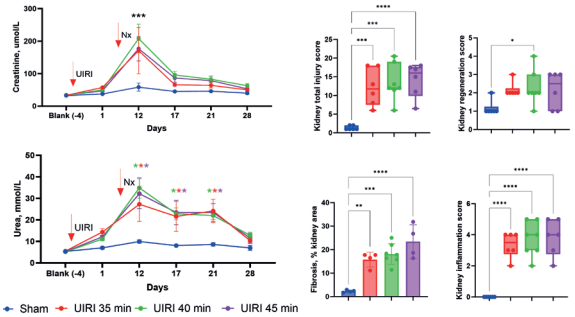


Figure 2. Serum Creatinine and Urea levels and Kidney total injury, regeneration, inflammation scores, and fibrosis-occupied area in UIRI-Nx rats throughout the study. *p<0.05, the color of the asterisk represents the comparison group (left panel), **p<0.01, ***p<0.001, ****p<0.0001 compared to Sham-operated group (right panel)

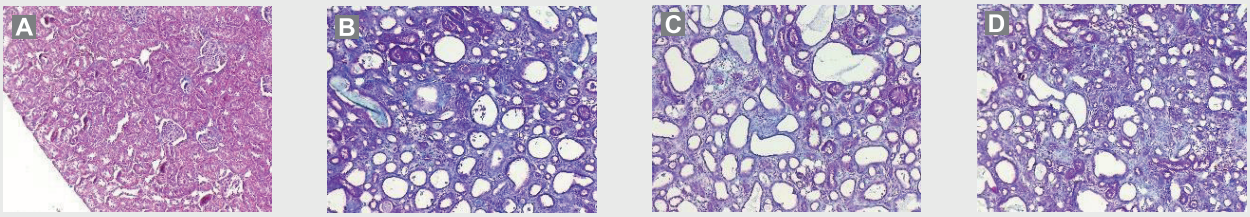


Figure 3. Microphotographs of injured kidney of rats 28 d post-UIRI; Masson's trichrome, Mg. x100. A – Sham, B – UIRI 35 min occlusion, C – UIRI 40 min occlusion, D - UIRI 45 min occlusion. Substantial area occupied with collagen fibers (fibrosis), colored with blue, is observed in B-D.

Conclusions

In this study, we successfully established a rat model for kidney ischemia-reperfusion injury, defining occlusion timeframes for various research objectives. For assessing kidney function post-treatment, occlusion times of 35-40 minutes are adequate, while longer occlusion times are recommended for studying fibrosis development.

Contact

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References

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2. Fu Y, Tang C, Cai J, Chen G, Zhang D, Dong Z. Rodent models of AKI-CKD transition. Am J Physiol Renal Physiol. **2018**;315(4):F1098-F1106