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Current perspective

Multiphoton imaging of kidney pathophysiology

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ABSTRACT

The number of people being diagnosed with end-stage renal disease is increasing globally. Therapeutic options to slow or halt the progression of kidney disease are limited and are not always successful, despite the increasing body of research and number of basic scientific reports in this field. Further studies are required to investigate new approaches to renal pathophysiology. State of the art optical imaging is a powerful tool used to non-invasively observe the pathophysiology of small animals and has the potential to elucidate the unknown mechanisms of renal disease and aid in our understanding of the disease. This paper is a brief summary of the current usefulness of intravital imaging using multiphoton microscopy and discusses possible future applications of the technique.

1. Introduction

Multiphoton microscopy is a powerful tool that can be used to analyze physiology *in vivo*. Although there were initial technical difficulties in observing peripheral organs, especially by using an upright microscope, several supporting devices have been developed and it is now easier to access the target organs. Additionally, developments in biotechnology have allowed for targeted cell fluorescence in mice and rats. While studies using these genetically modified mice and rats have shown the usefulness of intravital imaging to address a number of physiological questions, there are alternative techniques to visualize the physiology of animals. This article reviews the current availability of intravital imaging using multiphoton microscopy for the analysis of renal pathophysiology, such as acute kidney injury and glomerular permeability, especially focusing on techniques that do not use genetically modified mice, and also examines pharmacological experiments and discusses the future application of such methods for the treatment of kidney disease.

2. Acute kidney injury

Acute kidney injury (AKI) is a common clinical syndrome defined as the sudden onset of reduced renal function and characterized by a reduction in the glomerular filtration rate (GFR) and oliguria (1,2). Although basic research has identified several pathways involved in the mechanisms of AKI (3–6) which has enabled the development of new therapeutic strategies, specific treatments that can improve AKI outcome are not yet clinically available (7–9). Molitoris et al. from Indianapolis University are the first to introduce multiphoton *in vivo* imaging (10) as a useful tool to detect dynamic changes in the renal environment during AKI. Even though there are limits in accessibility, with observation being restricted to superficial areas (<100 μm from the surface), this technique, combined with the genetic engineering of mice, holds promise in exploring the hidden mechanisms behind AKI pathology and for evaluation of the efficacy of new therapeutic strategies (11).

Acute tubular necrosis, cell sloughing, and cast formation are typical histopathological changes observed in ischemic AKI. These pathological changes are often seen in the medullary region, but also can be observed in the cortical region using *in vivo* multiphoton imaging (Figs. 1 and 2, and Supplementary Movie 1, 2 and 3).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2016.08.001.

As seen in Fig. 1 and Supplementary Movie 1 and 2, we were able to observe both tubular cell sloughing and the subsequent migration of tubular cells in the wound healing process *in vivo*. It is classically hypothesized that the 'hole' in the proximal tubular monolayer after cell detachment closes with the migration of surrounding cells that have survived the trauma. To the best of our knowledge, our data are the first to confirm this hypothesis.

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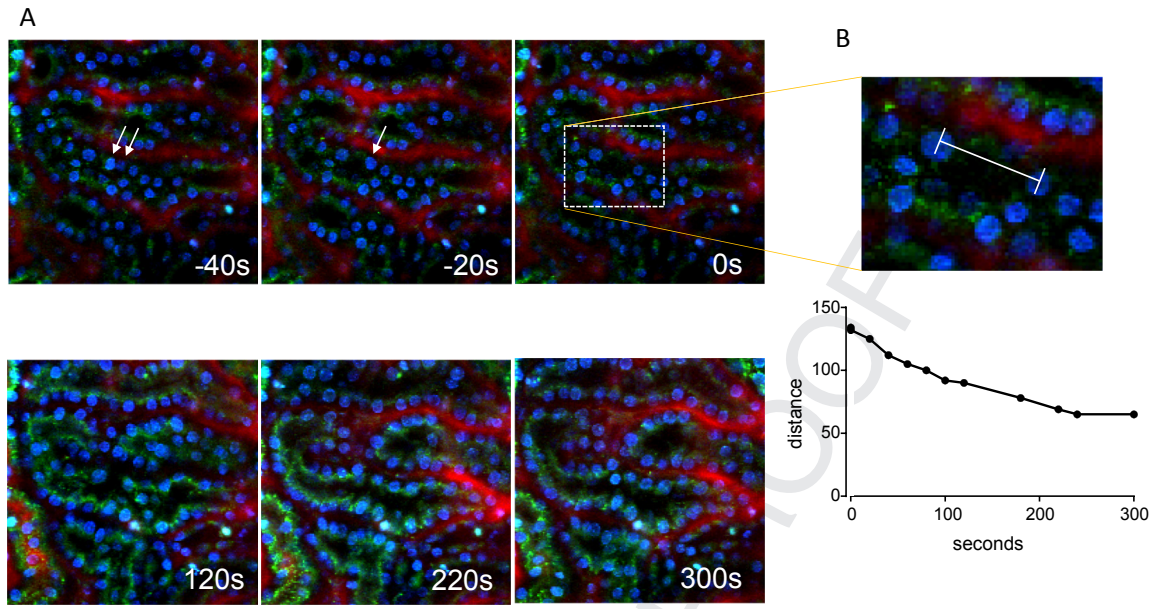

Fig. 1. Cell sloughing in the ischemia/reperfusion injury kidney. Cell nuclei were stained with Hoechst 33342 and plasma was stained with Evans blue-conjugated albumin at 24 h after reperfusion. The faint dot-like green fluorescence was derived from tubular auto-fluorescence, and the dark space between the green fluorescence represents the tubular lumen. (A) The two cells indicated by white arrows at −40 s were sloughed away at −20 s and 0 s. (B) The distance between the two nuclei of the adjacent cells in the enlarged image (right, top) began to shorten immediately after sloughing and stablized within 300 s. The image at 300 s (A) shows that the wound created by cell sloughing had healed through mitosis of the surrounding cells.

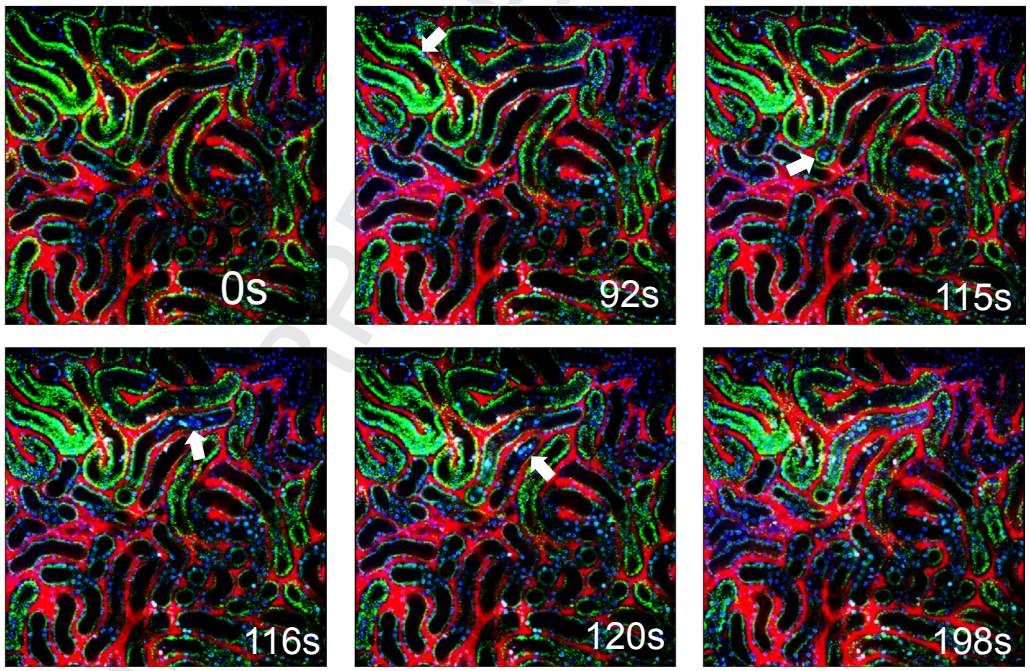

Fig. 2. Cellular cast formation in the ischemia/reperfusion injury kidney. Cell nuclei were stained with Hoechst 33342 and plasma was stained with Evans blue-conjugated albumin at 24 h after reperfusion. Strong fluorescence in S1 proximal tubules was derived from quinacrine. Arrows indicate: proximal tubular lumen dwindled at 92 s; some nuclei appeared in the tubular lumen at 115 s; nuclei flowed downstream of the tubule at 116 s; nuclei appeared in another tubule in the imaging window at 120 s; and nuclei created cellular casts at 198 s.

Surprisingly, cell sloughing quickly triggered the observed migration of cells, and the wound created by the loss of the two cells in the image healed within 300 s (Fig. 1B). This quick healing response may preserve the tubular monolayer after massive numbers of cell death even though it results in a shortening of the nephron length (12). We also observed that cellular cast formation could occur within 1 min (Fig. 2, and Supplementary Movie 3) but failed to observe other events such as clearance of debris (13,14) and repair of tubules with the proliferation of cells that survived (15). These events may take a longer time to occur in comparison with our observation time (24–25 h after ischemia/reperfusion injury), or occur deeper within the nephron, such as in the medullary region,

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