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Real-time 2-photon Intravital Kidney Imaging of Chronic Kidney Disease Mouse Model

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Objectives: Chronic kidney disease (CKD) is a condition that causes a gradual loss of kidney function over time. There are no specific symptoms until the disease progresses, making it difficult to make a quick diagnosis. Also, serial follow-up studies on the CKD pathway were rare. Therefore, we tried to understand the pathophysiological mechanism of CKD by non-invasively observing changes in renal pathology over time during the development and progression of CKD while mice were alive.

Methods: We first established a method for noninvasive observation of kidneys using two-photon intravital imaging in normal control and 0.2% adenine diet-induced CKD mouse models. Next, we set a strategy to repeatedly observe pathological changes from the early stages of CKD onset to later stages with severe fibrosis for 30 days in the same living mouse kidney. Furthermore, intravital imaging of CKD mouse kidney was compared with immunohistochemistry, periodic acid-Schiff, and Masson's trichrome stains.

Results: Using *in vivo* imaging, we observed abnormal morphology of the tubules, renal fibrosis, and numerous 2,8-DHA crystals deposited in the renal tubules. We could detect 2,8-DHA crystals from the 2nd day of induction of the adenine diet, and then tubular abnormalities and renal fibrosis were followed, which worsened over time. In this study, we established a method of observing the kidney using a two-photon intravital microscopy. The results of longitudinally observed *in vivo* images were correlated with pathological images obtained at the sacrifice of mice. These results also correlated with kidney function tests, including BUN and Cystatin C.

Conclusions: Real-time 2-photon intravital kidney imaging is an excellent research technique for studying the pathogenesis of CKD, as pathological changes can be continuously confirmed with an *in vivo* microscope without sacrificing the mouse every time.

Two-photon microscopy imaging of the mouse kidney.