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Pharmacophotonics: Utilizing multi-photon microscopy to quantify drug delivery and intracellular trafficking in the kidney ☆

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Abstract

The recent introduction of multi-photon microscopy coupled with advances in optics, computer sciences and the available fluorophores used to label molecules of interest have empowered investigators to study the dynamic events within the functioning kidney at cellular and subcellular levels. This emerging technique, with improved spatial and temporal resolution and sensitivity, enables investigators to follow the cell specific uptake of large and small molecules, determine the mode of cellular uptake, intracellular trafficking and drug metabolism in complex heterogeneous organs such as the kidney over time. Repeat determinations over seconds to hours to days allow for multiple observations within the same animal, thereby minimizing animal use and inter-animal variability. This can be particularly useful for preclinical studies. Furthermore, the ability to obtain volumetric data (3-D) makes quantitative 4-D (time) analysis possible. Finally, up to three fluorophores can be visualized simultaneously allowing for three different or interactive processes to be observed and resolved.

Keywords: Kidney; Multi-photon microscopy; Toxicology; Imaging; Pharmacology; Cell toxicity; Preclinical data

Contents

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1. Introduction

New imaging technologies, such as multi-photon microscopy, have equipped researchers with extremely powerful tools to uniquely address biologically important questions that can only be accomplished in whole organ studies [1–7]. In parallel with this, advances in fluorophores with increased quantum yields and ease of labeling [7–10], molecular and transgenic approaches, and new delivery techniques have allowed for the development of intravital studies that can follow and quantify events with enhanced spatial and temporal resolution and sensitivity at subcelluar levels [3,11–16]. Several previous publications have interrelated the different imaging modalities by showing their respective detection sensitivity and spatial resolution [17,18]. Multi-photon microscopy is uniquely positioned to compliment other in vivo imaging techniques that are limited in either resolution and sensitivity. However, multi-photon microscopy suffers from lack of tissue penetration, limiting its use in clinical situations. Exponential developments in computer sciences, specifically with applications to imaging, have removed many of the obstacles previously limiting the ability to utilize microscopy to study and quantify dynamic cellular processes [19–21]. In particular, developments in hardware, software, bandwidth and data storage now provide systems that possess the necessary speed to effectively and efficiently approach data intensive processes utilizing digital imaging analysis. These imaging technologies enable the dynamic measurement of four-dimensional (3-D plus time) structure in organs and isolated tissues [22,23], the measurement of chemical and biochemical composition of tissues and the expression of fluorescently labeled molecular agents including drugs and proteins. It also allows for quantification of the rates of physiological processes such as microvascular perfusion rates and the mechanism of cellular drug uptake and intracellular trafficking. This will greatly enhance the understanding of pharmacology, physiology and disease processes, and will hasten and improve the reliability and interpretation of preclinical data. The potential importance of imaging to the medical and pharmacology disciplines has been recently emphasized [18,24,25].

In this review, we will outline how we, and others, have utilized multi-photon microscopy to advance the understanding of physiologic and pathophysiologic processes within the kidney. Our main purpose is to outline and illustrate how this technique can be used to provide new and novel insights for drug delivery, uptake and intracellular trafficking.

2. The kidney and drug handling

2.1. Approach to complex heterogeneous organ

The kidney is an extremely complex and heterogeneous organ consisting of vascular and epithelial components functioning in a highly coordinated fashion that