A crosscorrelation methodology for *in vivo* pharmacokinetic study by the trans-scale fluorescent system

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Method Article

Keywords:

Posted Date: January 10th, 2022

DOI: https://doi.org/10.21203/rs.3.pex-1733/v1

Abstract

Conventional single-organ-isolation-based pharmacokinetics study is short of time-course information and exists considerable inaccuracy due to the inter-individual differences and characteristic imparities between *in vivo* and *ex vivo* tissues/cells. The *in vivo* time-course and multi-organs study of model drugs in living subjects could afford precise spatio-temporal correlation. Herein, a revolutionized trans-dimensional fluorescence system was home built, with the macro-level detection part for simultaneous pharmacokinetic study in different organs, and one confocal imaging needle for micro-level visualizing cellular uptake of drugs with super-high resolution (0.472 μm). Correlating these simultaneous acquired trans-scale data, an innovative physiologically-based pharmacokinetics (PBPK) model was firstly created for predicting drug disposition in other species. Its accuracy and reliability was firmly supported by the high consistent predicted-data with the real-measured data in mice and in human, respectively. This study provides an innovative methodology and revolutionized instrument for *in vivo* real-time advancing assessment of druggability.

Introduction

Reagents

Equipment

Procedure

The trans-scale fluorescent system and optical characterization

The trans-scale fluorescent system consisted of two parts: fluorescence intensity detection and confocal imaging part. The fluorescence intensity detection part was mainly composed of three Y-shaped optical fibers and a spectrometer, which was used for real-time trans-scale continuous monitoring of the fluorescence intensity of different organs. The confocal imaging part with super resolution was well used for *in vivo* cellular level imaging, owing to the flexible and convenient optical fiber needle and high-performance confocal laser scanning system.

The sensitivity of fluorescence intensity detection part was calibrated by fluorescein with different concentration gradients in phosphate buffered solution (PBS) containing 1% DMSO (v/v). Besides, fluorescence intensities of blood and different organs homogenate (liver and kidney) treated with fluorescein of different concentrations were detected by Y-shaped optical fibers. When the blood and organ homogenate were performed, they had been covered with a layer of vessels and serous membrane, respectively. The optical performance of the confocal imaging part was characterized by measuring the resolution using 100 nm sized fluorescent beads (Excitation/Emission: 488 nm/530 nm), embedded in 1% agarose. All measurements were based on the FWHM of the intensity profiles. The lateral resolution was deduced from an x-y scan of the focal plane wherein several beads were imaged. An intensity profile

across each bead was extracted and the averaged FWHM across all beads in the frame was taken to be the lateral resolution. The 100 nm fluorescent beads were tomographic imaged each time after mechanically moving the beads by 1 μm per step. The intensity profile was extracted across the axial cross-sectional view of the fluorescent beads in the x-z and y-z scan and the FWHM of the Gaussian profile was estimated to be the axial resolution.

Software control

The trans-scale monitoring system software was written using a combination of Labwindows and MATLAB tools. This program integrated several functional modules such as laser source control, signal display and analysis, control timing sequence and data acquisition. Confocal image data was acquired by ScanImage software.

Experimental animals and ethics statement

ICR mice with body weight 20-25 g, Balb/c nude mice with body weight 18-22 g and SD rats in weight of 180-200 g were purchased from Nanjing Qing Longshan Laboratory Animal Technology Company. The animals were fed a laboratory diet with water and food and kept under constant environmental conditions, with 12 h light/dark cycles. All animal study was performed in compliance with the Animal Management Rules of the Ministry of Health of the People's Republic of China (document No. 55, 2001) and the guidelines for the Care and Use of Laboratory Animals of the China Pharmaceutical University. ICR mice were used as the model mice for the liver and kidney damage. The SD rats and BALB/c nude mice were used for the drug pharmacokinetic study. n = 3 mice/group.

Cell culture

Human liver cancer cells HepG2 and human normal liver cells L02 were maintained in RPMI-1640 (Solarbio, China) complete medium (which supplemented with 10% FBS (Gibco) and100 μg/mL penicillin, and 100 μg/mL streptomycin) in a 5% CO2 humidified atmosphere at 37°C.

Mouse model of orthotopic liver cancer

Ten BALB/c nude mice (male: female = 1:1) were used in this study. The mouse liver was exposed by laparostomy, and suspension of HepG2 cells (1×10^5 cells/mouse) were injected into the left lobe of liver for preparation of a mouse model of orthotopic liver cancer.

Mice models of liver fibrosis and kidney injury

ICR mice (20-25 g) were randomly assigned to four groups, including liver fibrosis[1], kidney injury and two different control groups. The mice for liver fibrosis model were subjected to the following process. While the mice were on a high-sugar and high-fat diet, 10% CCl_4 diluted olive oil solution (5 ml/kg) was administrated subcutaneously every three days, and the administration cycle for mice with mild, moderate and severe liver fibrosis was 7, 30 and 60 days, respectively. The pure solvent olive oil served as control. For kidney injury model[2,3], the animals were intraperitoneally injected with cisplatin solution (2 mg/kg) every three days for six time and the phosphate buffered solution (PBS) served as control group.

In vivo Imaging

Throughout the entire session, the animals were anesthetized using an isoflurane inhalation anesthesia system (RWD, China). The subjected organ (liver, kidney or cancer) was exposed through minimally invasive surgery in the abdominal cavity of the animal. The organ was gently wiped off and covered with a thin plastic wrap to prevent the needle from contaminating. The model drugs were injected through the tail vein. Subsequently, the organ was imaged *in vivo*.

Pharmacokinetics study

The subjected animals were continuously anesthetized with isoflurane gas throughout the experiment. The rat carotid artery intubation technique was used to collect blood sample at different time points for *ex vivo* detection as the traditional control. At the same time, the three Y-shaped optical fiber needles were placed directly above the organs, usually including vasculature, liver, tumor and kidney. The imaging needle was placed above the different organs of the same animal to collect imaging. The blood samples collected *ex vivo* were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS)[4] using methods such as centrifugation and extraction to obtain the substance concentration at each time point. Finally, the pharmacokinetic analysis was performed by ADAPT 5 with NLEM method.

Pathological section staining

Morphological observation was assessed by H□E staining as previously described[5]. Briefly, after euthanized, the organs were carefully excised from the animals and immediately fixed, and the organs were cut into appropriate size and fixed in 4% neutral paraformaldehyde solution at room temperature for 48 h, dehydrated with different concentration gradients of ethanol and xylene, and then embedded in

paraffin using the Microm STP 120 from Leica. Pathological sections (3-4 μm) were cut using the Microm HM 325 (Leica, Germany), dried onto strongly adhesive at 55 ℃, rehydrated in ethanol and stained with the corresponding staining kits. H&E staining was used to evaluate the histological morphology. Similar procedures were performed in Masson staining. Finally, Sections were captured the picture with a light microscope.

MTT assay and colony formation assay

L02 cells were seeded in 96-well plates (NEST Biotechnology, China) at a concentration of 2.0-4.0×103 cells per well, followed by treatment with serial dilutions of dye R3 (0.01-0.3mg/mL) and incubated for 12h, 36h and 72h separately. Cell proliferation viability was assessed by MTT reagent (5mg/ml, 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, Solarbio).

Similarly, L02 cells, seeded in a six-well plate, were treated with 0, 0.1, 0.3 and 0.5 mg/mL R3 dye for 7 days, afterwards stained with crystal violet solution (0.5% in 20% methanol). Images were taken by a Nikon camera. The results are reported as the mean ± standard deviation (SD) of three independent experiments. Quantitative result graphs were analyzed with GraphPad Prism 7.0 software.

Acute toxicity test

ICR mice were divided into two groups (n=3 mice/each group). After 8 h of starvation treatment, dye R3 dissolved in normal saline (90 mg/kg) were injected into the tail vein, and the mice in control group were given normal saline. Body weights of mice were recorded every days and the organs (heart, liver, spleen, lung, and kidney) were applied to H□E staining after 14 days observation.

Statistics

The data is represented by the mean ± SD. Statistical analysis was performed using student t test for two group comparison and One-Way ANOVA for more than two groups. $P<0.05$ was considered statistically significant.

Troubleshooting

Time Taken

Anticipated Results

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Acknowledgements

We acknowledge financial support from the National Natural Science Foundation of China (NSFC 81727804, 91859204, 81729002 and 81801816) and Natural Science Foundation of Jiangsu Province (SBK2018041202).