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A simple blood microdialysis in freely-moving rats for pharmacokinetic–pharmacodynamic modeling study of Shengmai injection with simultaneous determination of drug concentrations and efficacy levels in dialysate

Shuyu Zhan[a,*], Baoyue Ding[a,*], Yu-er Ruan[a], Xuan Huang[a], Guoqiang Liu[a], Xiaoqing Lv[a], Xiong Huang[a], Mingjuan Li[a], Ninghua Jiang[b], Qing Shao[c]

[a] Department of Pharmaceutics, Medical College of Jiaxing University, Jiaxing 314001, China
[b] Department of Pharmacy, Jiaxing Second Hospital, Jiaxing 314000, China
[c] Pharmaceutical Informatics Institute, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

Microdialysis is a powerful *in vivo* sampling technique for pharmacokinetic–pharmacodynamic (PK-PD) modeling of drugs in pre-clinical and clinical studies. However, the noticeable limitations of previous studies using microdialysis were that animals anesthesia in the whole experiment and the combination of microdialysis and blood sampling for drug and (or) effect detection, which can obviously influence PK and PD behavior of drugs. In this study, a simple blood microdialysis sampling system in freely-moving rats was established for simultaneous study of PK and PD of Shengmai injection (SMI) effect on inducing real-time nitric oxide (NO) release on isoproterenol (ISO) induced myocardial ischemia rats. The LC–MS/MS and HPLC with fluorescence detection (HPLC-FLD) methods were developed to determine ginsenside Rg1, Rg2, Re, Rf, Rb1, Rd and Rc, the main effective components of SMI, and NO_x^-, the main oxidation products of NO, in dialysates respectively. Through simultaneous determination of drug concentrations and NO efficacy levels in dialysate, the developed methods were successfully applied to set up concentration-time and effect-time profiles followed by PK-PD modeling of SMI effect on inducing NO release after intravenous administration of 10.8 mL kg^{-1} SMI in myocardial ischemia rats. The PK-PD modeling characterized the dose-effect relationships of SMI and behaved good prediction ability. The established blood microdialysis in freely-moving rats is an appealing technology for rational PK-PD studies when selecting suitable blood endogenous micromolecule as effect marker.

1. Introduction

Shengmai injection (SMI), a famous traditional Chinese medicine composed of *Panax ginseng*, *Ophiopogon japonicas* and *Schisandra chinensis*, is widely used in China for the treatment of cardiac emergencies, such as coronary atherosclerotic cardiopathy, viral myocarditis and myocardial infarction [1–3]. In recent years, the pharmacological mechanisms and pharmacokinetic characteristics of SMI have been well studied [4–7], however, no study is available concerning its dose-effect relationship up to now, and the clinical administration depends only on clinical experience. Pharmacokinetic–pharmacodynamic (PK-PD) modeling is a powerful tool to understand underlying pharmacological mechanisms and optimize dosage regimens through characterizing the dose-effect relationship of drugs, and it has been widely used in pre-clinical and clinical studies of drugs [8]. Recently, owing to quality control questions and sightless clinical applications, a significant number of severe adverse drug reactions cases of traditional Chinese medicine injections, including SMI, have been reported. Hence, to enhance clinical efficacy and safety of SIM, it is important to characterize the dose-effect relationship of SMI in pre-clinical and clinical studies through PK-PD modeling.

Microdialysis is a powerful *in vivo* sampling technique to measure unbound (free) drug concentrations or endogenous substances over time in extracellular fluid from virtually any tissue in the body, including the blood, brain, skin and so on. The technique uses a microdialysis probe containing a semi-permeable membrane which is inserted into the selected tissue followed by continuous perfusion with a suitable perfusate. Small molecules will be transported over the semipermeable membrane through passive

* Corresponding authors.
E-mail addresses: zhansy2000@163.com (S. Zhan), lena_310@163.com (B. Ding).

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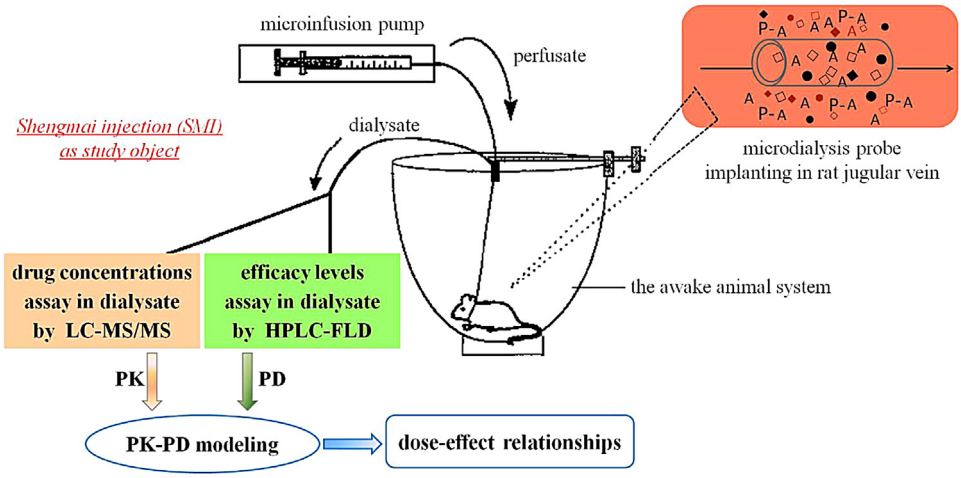

Fig. 1. A blood microdialysis in freely-moving rats for PK-PD modeling study of SMI.

diffusion, while larger bio-macromolecules are excluded. As a consequence, microdialysis samples generally can directly be used to analysis [9]. Microdialysis sampling is minimally invasive, offers the possibility of real-time sampling and since no fluid loss occurs, long term sampling is possible without interfering with the pharmacokinetic behavior of the drug [10]. Therefore, microdialysis is very suitable for pharmacokinetic, pharmacodynamic and integrated PK-PD studies [11]. Nevertheless, in spite of its appealing advantage and wide application, there are still some limitations for the technology of microdialysis sampling. One of noticeable limitations is most studies, especially for PK-PD modeling, using microdialysis sample were performed on anaesthetized animals because of technical bottleneck. Höcht et al. [12] designed a "shunt" microdialysis probe with one vascular inlet and two vascular outlets to study PK-PD properties of some antihypertensive agents in anaesthetized hypertensive rats, which used the inlet and one vascular outlet of the probe for examining the time course of the plasma concentration of drugs, and meanwhile the remaining vascular outlet was connected to a pressure transducer for mean arterial pressure (MAP) and the heart rate (HR) detection. Based on this technical probe, they elucidated the PK-PD properties and effect function characteristic of some drugs such as metoprolol [13], diltiazem [14], and verapamil [15]. However, if anesthesia in the whole experiment influences pharmacokinetic and pharmacodynamic behavior of cardiovascular drugs might remain to be investigated because earliest study had suggested anesthesia could profoundly perturb the pharmacokinetics behavior of drugs [16]. In addition, to perform simultaneous pharmacokinetic and pharmacodynamic studies, some researches combined microdialysis with blood sampling for separate drug and (or) effect marker detection [17,18], which obviously brought about larger trauma and fluid loss of animals compared with simple microdialysis sampling. Therefore, it is significant to establish simple blood microdialysis system with freely-moving rats for rational PK-PD modeling studies.

The aim of this study is to set up a blood microdialysis system in freely-moving rats for simultaneous pharmacokinetic and pharmacodynamic profiles followed by establishing PK-PD modeling of SMI effects on isoproterenol (ISO) induced myocardial ischemia rats. For many PK-PD modeling, it is critical to screen suitable effect biomarker which quantitatively relates to the dose and causally links to clinical outcomes [19], and most importantly, can be simply sampling. Modern pharmacological studies indicated that ginsenosides, the main effective components of SMI, dilated coronary vessels, which increased coronary flow and protected heart from myocardial ischemia injury by up-regulating endothelial nitric oxide synthase and inducing nitric oxide (NO) release in endothelial cells [20,21]. Thus, NO may be an available blood biomarker reflecting the effect of drugs on myocardial ischemia. In our previous study, we had validated that ginsenside Rg1 and Rb1 can inducing real-time NO release in ISO treated rats compared with control and model rats [22]. Therefore, in this study, selecting NO_2^- and NO_3^- (NO_x^-), the main oxidation products of NO in blood, as effect marker and the main effective components of SMI, ginsenside Rg1, Rg2, Re, Rf, Rb1, Rd and Rc, as pharmacokinetic marker, we tried to investigate the dose-effect relationships of SMI effect on inducing real-time NO release in ISO treated myocardial ischemia rats using a blood microdialysis system in freely-moving rats (Fig. 1).

2. Experimental

2.1. Chemicals and reagents

Ginsenoside Rg1 (Rg1), ginsenoside Rg2 (Rg2), ginsenoside Re (Re), ginsenoside Rf (Rf), ginsenoside Rb1 (Rb1), ginsenoside Rd (Rd) and ginsenoside Rc (Rc) were purchased from Shanghai Winherb Medical S&T Development Co. Ltd (Shanghai, China). Shengmai injection was supplied by CHANGSHU LEI YUN SHANG Pharmaceutical Limited (suzhou, Jiangsu, China). Isoproterenol (ISO), 2,3-diaminonaphthalene (DAN), nitrate reductase from Aspergillus niger, flavin adenine dinucleotide disodium salt hydrate (FAD), and lactic dehydrogenase (LDH) from Lactobacillus leichmanii were purchased from Sigma. β-Nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Beyotime (Beyotime Institute of Biotechnology, China). Digoxin (internal standard, IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile, methanol (Merck, Germany) and acetic acid (Tedia, USA) were of HPLC grade. Water was produced using a Milli-Q water system (Millipore, Bedford, MA, USA). All the other reagents were obtained from commercial sources and were of analytical grade.

2.2. Animals

Adult, male Sprague-Dawley rats, weighing 230–270 g, were bought from the Experiment Animal Center of Zhejiang Province (Hangzhou, China). The rats were housed in a temperature-controlled room (with temperature of $22 \pm 2\,^{\circ}C$ and relative humidity of $50 \pm 10\%$) under a 12 h light-dark cycle with free access to food and water. The animal experiments were performed in