Journal of Pharmaceutical and Biomedical Analysis 96 (2014) 231–240

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development of a microdialysis system to monitor lamivudine in blood and liver for the pharmacokinetic application in herbal drug interaction and the gene expression in rats

Chia-Ming Lu[a], Mei-Ling Hou[a], Lie-Chwen Lin[b], Tung-Hu Tsai[a,c,d,*]

[a] Institute of Traditional Medicine, National Yang-Ming University, Taipei, Taiwan
[b] National Research Institute of Chinese Medicine, Taipei, Taiwan
[c] Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan
[d] Department of Education and Research, Taipei City Hospital, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 31 March 2014
Accepted 1 April 2014
Available online 12 April 2014

Keywords:
Herbal medicine
Lamivudine
Microdialysis
Pharmacokinetics
Traditional Chinese medicine

ABSTRACT

The aim of study is to develop a novel multiple microdialysis technique coupled to a validated chromatographic system for the measurement of protein-unbound form lamivudine and investigation of its herb–drug interaction in rat blood and liver. Furthermore, gene expression changes of drug metabolizing enzymes in rat were evaluated by microarray analysis after being treated with a traditional Chinese herbal formulation, Long-Dan-Xie-Gan-Tang (LDXGT). The analyte was separated by a reverse-phase C18 column using the mobile phase comprising methanol and 10 mM KH_2PO_4 (15:85, v/v, adjusted to pH 6.0 with NaOH) with the flow rate of 0.8 mL/min, and the UV wavelength was set at 270 nm. The processes of method validation followed Food and Drug Administration (FDA) guidelines. The pharmacokinetic data demonstrated that the area under the concentration–time curve (AUC) of the lamivudine alone and the LDXGT pretreated group were 532 ± 37.6 and 550 ± 44.2 min μg/mL in rat blood after lamivudine administration (10 mg/kg, i.v.) and 682 ± 196 and 642 ± 153 min μg/mL in rat liver, respectively. The herb–drug pharmacokinetic interaction showed that with either lamivudine alone or in combination with pretreated with LDXGT, the pharmacokinetic parameters were not significantly changed except the apparent volume of distribution (V_d) at a high dose of lamivudine (30 mg/kg). In addition, microarray analysis showed that among 70 altered genes (selection criteria: |Fold change| ≧ 2 and $p < 0.05$), only 11 genes were involved in drug metabolism and indicated that a relatively small portion of drug metabolizing genes in liver were altered at the genome level after the therapeutic dose of LDXGT treatment. In conclusion, these studies provide constructive information to interpret the herb–drug interactions between lamivudine and a popular Chinese herbal formulation.

1. Introduction

Lamivudine (2′,3′-dideoxy-3′-thiacytidine, commonly called 3TC) is an antiretroviral drug used worldwide for the treatment of hepatitis B virus infection [1,2]. It was the first drug that is a nucleoside analog and reverse transcriptase inhibitor to have been approved by the FDA for HBV infection in 1998, and in 1999 it was subsequently approved by the Department of Health for HBV infection treatment in Taiwan [3].

Although the advantages of using the standard Western medicine drugs for chronic viral hepatitis therapy are clear, a number of patients (about 38–42% of the global chronic hepatitis population) are still seeking helps from complementary and alternative medicine (CAM) [4–6]. However, safety issues concerning potential side effects and toxicity of herbal products have not been adequately addressed to date. Thus, assessment of the safety of herbal plants and herbal dietary supplements is timely and important [7,8].

According to a survey from the Database of National Health Insurance Research in Taiwan, the herbal formulation of Long-Dan-Xie-Gan-Tang (LDXGT) is the most frequently used in the treatment of chronic hepatitis [6]. Since many people in Taiwan take LDXGT and lamivudine together for chronic hepatitis treatment, it is important to investigate the potential herb–drug interaction of LDXGT and lamivudine.

* Corresponding author at: Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, 155, Li-Nong Street Section 2, Taipei 112, Taiwan. Tel.: +886 2 2826 7115; fax: +886 2 2822 5044.
E-mail address: thtsai@ym.edu.tw (T.-H. Tsai).

http://dx.doi.org/10.1016/j.jpba.2014.04.001
0731-7085/

Recently, some analytical methods have been reported for lamivudine determination in vivo, and the conventional methods for sample preparation of lamivudine from biological samples is commonly performed with protein precipitation, liquid–liquid extraction or solid phase extraction [9,10]. However, these methods are time-consuming and require tedious preparation prior to HPLC analysis. In contrast to other biological sampling methods, microdialysis technique provides a very clean dialysate, which requires no further cleaning process. In addition, microdialysis is a useful sampling technique for monitoring protein-unbound substances consecutively at target sites in vivo without excessive bodily fluid loss from the experimental animals, and it increases temporal resolution for pharmacokinetic studies. Only free-form drugs are physiologically available for drug distribution to the target sites and therapeutic applications [11]. Furthermore, sampling by microdialysis reduces the number of animals used for distribution studies. Hence, microdialysis method was chosen in this study due to these advantages.

Pharmacologically, herbal formulas are multi-component, multi-mechanism, and multi-target. Thus to establish the gene expression profiles of an herbal formula using systematic tools may be an efficient method for investigating both the mechanism of the herbal formula and the relationship with its herbal components [12]. There have been studies investigating the alterations of gene expression after ingesting herbal medicine or herb extract using microarray analysis [13,14]. For examples, the results of Cheng et al. [15] demonstrate that the gene expression profiles change in the liver and kidney of mice after oral administration with herbal formulae for 7 consecutive days. In addition, by pathway analysis, more than 90% of biological pathways are regulated by formulae. Guo et al. (2009) [16] previously investigated the alterations in gene expression of drug metabolizing enzymes in the livers of Fischer 344 rats administered kava extract for 14 weeks. The results indicate that kava extract altered the expression of CYP 1A1 (Cytochrome P450 1A1) and other CYP enzymes, and many genes had been changed. In addition, another study shows that some important information may be missed if the whole spectrum of gene expression is not obtained, and the gene expression profiles in the livers of rodents treated with herbal dietary supplements using microarray analysis is a potentially practical approach for understanding the mechanism of toxicity [17]. In the current study we used LDXGT to illustrate the gene expression profiles by microarray analysis.

To our knowledge, this is the first study on the pharmacokinetics of lamivudine using microdialysis sampling, and there has been no investigation into the herb–drug interaction of LDXGT and lamivudine. The aim of this study is to develop a microdialysis technique coupled to a validated HPLC-UV method for measuring free-form lamivudine in rat blood and liver, and subsequently to examine the pharmacokinetic herb–drug interactions of LDXGT on lamivudine. Moreover, the gene expression of drug-metabolizing enzymes in the livers of Sprague Dawley rats administered LDXGT by gavage for 5 days was examined in this study. This study may provide a useful model of pharmaceutical research for future application and contribute to the understanding of the herb–drug interactions between Chinese herbal formulations and common Western drugs.

2. Materials and methods

2.1. Chemicals and reagents

Lamivudine was purchased from the United States Pharmacopeia (USP, Rockville, MD, USA). Urethane and α-chloralose were obtained from Sigma–Aldrich Chemicals (St. Louis, MO, USA). HPLC grade methanol, citric acid, sodium citrate, dextrose, sodium chloride, potassium dihydrogen phosphate (KH_2PO_4), orthophosphoric acid (H_3PO_4, 85%) and sodium hydroxide were purchased from E. Merck (Darmstadt, Germany). Deionized water was prepared by Millipore (Milford, MA, USA) and used for all preparations in this study.

The pharmaceutical herbal product LDXGT manufactured in accordance with Good Manufacturing Practice (GMP) for Chinese crude drugs was obtained from the Sheng Chang Pharmaceutical Co., LTD. (Taipei, Taiwan) and has been used medicinally for patients. The pharmaceutical herbal product contained the following ingredients: *Gentiana scabra* (4.0 g), *Scutellaria baicalensis* (2.0 g), *Gardenia jasminoides* (2.0 g), *Alisma plantago* (4.0 g), *Akebia trifoliate* (2.0 g), *Plantago asiatica* (2.0 g), *Angelica sinensis* (2.0 g), *Rhemannia glutinosa* (2.0 g), *Bupleurum chinense* (4.0 g) and *Glycyrrhiza uralensis* (2.0 g). The above crushed herbs were extracted to 5.5 g, so the extraction yield of the decoction was about 21% (5.5: 26.0, w/w). Finally, each 9.0 g of LDXGT extract contained starch: concentrate herbal decoction (3.5: 5.5, w/w).

2.2. Experimental animals

All experimental protocols involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of National Yang-Ming University, Taipei, Taiwan (IACUC Approval No: 1020703). Male Sprague Dawley rats (220–280 g, 6–7 weeks of age) were obtained from the Laboratory Animal Center at National Yang-Ming University, Taipei, Taiwan.

2.3. Herbal formula pretreatment and drug administration

The dose of herbal formula for translation from human to animal is recommended by the US Food and Drug Administration guidelines as the following conversion equation: Human equivalent dose (HED, mg/kg) = animal dose (mg/kg) × (animal K_m/human K_m) [18,19].

The parallel study design was divided into the groups of lamivudine alone (10 or 30 mg/kg, i.v.) and the LDXGT-pretreated (1.23 g/kg, p.o., for 5 days). After the final dose of LDXGT on the fifth day, lamivudine (10 or 30 mg/kg) was injected into the femoral vein for microdialysis sampling.

2.4. Microdialysis experiments

The microdialysis system consisted of a CMA/400 microinjection pump, a CMA/142 microfraction collector (CMA, Stockholm, Sweden) and microdialysis probes. The microdialysis probes for blood and liver sampling were made in our laboratory [11]. The silica capillary was designed for a concentric shape and the tip of the probe was covered with a dialysis membrane (molecular weight cut-off of 13,000 Da, Spectrum, Laguna Hills, CA, USA) of 10 mm in length.

After one hour from the final dose of LDXGT, a rat was anesthetized intraperitoneally with mixture of urethane (1 g/mL) and α-chloralose (0.1 g/mL) at 1 g/kg, a polyethylene tube (PE-50; Clay Adams, NJ, USA) was inserted into the femoral vein for further drug administration. The blood microdialysis probe was positioned in the jugular vein toward the right atrium and perfused with anticoagulant citrate dextrose (ACD) solution consisting of citric acid 3.5 mM, sodium citrate 7.5 mM, and dextrose 13.6 mM. The liver microdialysis probe was implanted in the median lobe of the liver and also perfused with ACD solution [20]. The flow rate of ACD solution was set at 2.0 μL/min by a microinjection pump for blood and liver microdialysis. The surgical period lasted between 30 and 40 min. After the probe implantation and following a two hour post-surgical stabilization period, lamivudine (10 or 30 mg/kg) was administrated intravenously via the femoral vein. The sampling