Preclinical Pharmacokinetics of Lamivudine and Its Interaction with Schisandra chinensis Extract in Rats

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ABSTRACT: Schisandra chinensis (Turcz.) Baill. (S. chinensis) extract and its active ingredient, schizandrin, have been used as a botanical medicine and dietary supplement for the treatment of hepatitis. Lamivudine is an antiretroviral drug and is used to treat hepatitis B viral infection. The aim of this study was to develop an ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for the measurement of lamivudine and to determine the pharmacokinetic behaviors of an aqueous–ethanol extract of S. chinensis in rats. The separation was performed on a phenyl column maintained at 40 °C. The experimental animals were distributed into three groups: (1) lamivudine alone (10 mg/kg, i.v.); (2) lamivudine (10 mg/kg, i.v.) + pretreatment with S. chinensis (3 g/kg, p.o.); and (3) lamivudine (10 mg/kg, i.v.) + pretreatment with S. chinensis (10 g/kg, p.o.). The experimental results indicated that neither treatment with lamivudine alone nor pretreatment with S. chinensis (3 or 10 g/kg) significantly changed the pharmacokinetic parameters. In conclusion, based on the above preclinical experimental model, the combination of lamivudine with the herbal extract of S. chinensis did not exhibit significant pharmacokinetic interactions. These data offer useful information for assessing the preclinical safety of nutritional supplementation with lamivudine.

1. INTRODUCTION

The first reverse transcriptase inhibitor and nucleoside analog (2′,3′-dideoxy-3′-thiacytidine, lamivudine) for the therapy of hepatitis B viral infection was approved by the United States Food and Drug Administration (FDA) in 1998; the next year, the Department of Health in Taiwan approved the same drug for the treatment of hepatitis B virus (HBV) infection. A search of PubMed for botanical-derived drugs for the treatment of chronic liver disease confirmed that single-herb medicines, individual bioactive ingredients, and pharmaceutical preparations of Schisandra chinensis, silaymarin, and Salvia miltiorrhiza are often used by those suffering from chronic hepatitis. S. chinensis is a well-known traditional medicine and supplement that has been widely used in Asia and Europe, due to its hepatoprotective effects, for the treatment of human immunodeficiency virus, and as an antioxidant. Recent research has shown that dietary supplementation is commonly used for the promotion of health or for disease prevention. Therefore, dietary supplements containing S. chinensis extract are becoming increasingly popular in many Western cultures. In the United States, extracts from S. chinensis and Schisandra sphenanthera are usually incorporated into multicomponent dietary supplement preparations. Therefore, it is important to explore the interactions between dietary supplements containing S. chinensis and clinical medicines. Lamivudine and S. chinensis are commonly used for the treatment of hepatitis in Western and Eastern medicines, respectively. However, few reports describe the interactions of these two drugs from a pharmacokinetics perspective.

Several divergent analytical methods have been developed to measure lamivudine in pharmaceutical products and biological samples. The most widely used analytical techniques to assess lamivudine levels are high-performance liquid chromatography with ultraviolet detection (HPLC–UV), tandem mass spectrometry (LC–MS/MS), high-performance thin-layer chromatography (HPTLC), capillary electrophoresis, and liquid chromatography–high-resolution mass spectrometry (LC–HRMS). In addition, several analytical matrices have been studied, such as pharmaceutical tablets, human plasma, saliva, cerebrospinal fluid, human hair, human breast milk, dried blood spots, plasma, amniotic fluid, and rat tissues.

To assess the novelty of this work, a search of the PubMed database was conducted, and it showed that no pharmacokinetic investigations of the interaction between lamivudine and S. chinensis have been reported. In addition, based on the consumption of S. chinensis as a food product, we hypothesize that the herbal extract of S. chinensis at various doses would have only minor pharmacokinetic interactions. To investigate this
hypothesis, a UHPLC–MS/MS method for measuring lamivudine was developed, validated, and used to assess herb–drug pharmacokinetic interactions between lamivudine and *S. chinensis* in rats. The experimental rats were divided into the following three groups: (1) lamivudine alone (10 mg/kg, i.v.); (2) lamivudine (10 mg/kg, i.v.) + pretreatment with *S. chinensis* (3 g/kg, p.o.; this dose is equivalent to a dose of schizandrin of 5.2 mg/kg); and (3) lamivudine (10 mg/kg, i.v.; this dose is equivalent to a dose of schizandrin of 17.3 mg/kg) + pretreatment with *S. chinensis* (10 g/kg, p.o.).

### 2. RESULTS AND DISCUSSION

#### 2.1. Establishment of UHPLC–MS/MS Conditions

To assess the mass spectral fragmentation pattern of lamivudine, stock solutions of lamivudine (100 ng/mL) were analyzed by direct injection into the spectrometer. The mass spectral peaks of lamivudine and an internal standard at *m/z* 230.04 and 226.12 correspond to [M + H]+. The product ions, *m/z* 111.93 and 76.91, of these two analytes were selected for quantification, as shown in Figure 1A,B.

Regarding the analytical conditions, the positive mode was used for analyte identification. To optimize the separation of the analytes, reversed-phase C18 and C8 columns were considered. However, shoulders or tailing were seen in the chromatograms; therefore, an end-capped phenyl column was selected, which may be more suitable due to the hydrophillicity of the analyte.

Lamivudine and the internal standard were detected in the positive ion mode, and their retention times were approximately 2.5 and 2.3 min, respectively (Figure 2). Figure 2 shows the chromatogram of the extract of blank plasma obtained by solid-phase extraction (SPE), and it indicated that the signal peaks from the biological sample would not be affected by endogenous interferences. With the exception of the analyte peaks, no chromatographic peaks were detected in the plasma of untreated rats. Figure 2 also shows a chromatogram of blank plasma spiked with lamivudine (10 ng/mL) and the internal standard (5 ng/mL). These results revealed that the method offers acceptable selectivity for the identification of lamivudine in biological samples.

The detection of analytes in the positive ion mode via the monitoring of the precursor–product association in the multiple reaction monitoring (MRM) mode showed good sensitivity and intensity for analysis of the target component. In this research, the selected reaction monitoring transition was *m/z* 230.04 → 111.93 for lamivudine, which was consistent with previous research. The spectrometry conditions such as the analytical column and the components and the ratio of the mobile phase were optimized to achieve good peak shapes and intensities. Under the optimized conditions, the retention times of lamivudine and methyl yellow were 2.5 and 2.3 min, respectively. The results showed that the peak shape in methanol was better than in other solvents, and therefore, methanol was chosen as the organic solvent. To optimize the intensity and peak shape and avoid tailing, 0.1% aqueous formic acid was used.
as the aqueous component of the mobile phase. Finally, a methanol−0.1% aqueous formic acid solution was used as the mobile phase in this study.

In our study, we developed an improved UHPLC−MS/MS method for studying the effect of *S. chinensis* on the pharmacokinetics of lamivudine in rats. Previously, an HPLC−UV19 method was reported for the measurement of lamivudine in rat plasma; however, this method requires a larger biological sample volume and more time, and the sample preparation is laborious. In addition, another reported HPLC−MS/MS method20 had a total run time of 8 min, while ours was no more than 5 min. In addition, the determination of the lamivudine concentration in rat plasma is more accurate, facilitating pharmacokinetic studies.

2.2. Method Validation of Lamivudine in Rat Plasma.

The standard profile was established by the peak−area ratios of lamivudine to the internal standard. The standard curve for lamivudine was \( y = 0.0023x + 0.001 \). The coefficient of correlation for each standard profile was >0.995. The data indicated that the linear concentration range of lamivudine was 5−1000 ng/mL. The results demonstrated good reproducibility. The limit of detection (LOD) and lower limit of quantitation (LLOQ) for lamivudine were 1 and 5 ng/mL, respectively. The bias and the relative standard deviation (RSD) were evaluated at concentrations of 5−500 ng/mL for lamivudine. These data are shown in Table 1.

The LLOQ and LOD are defined as the concentration at which the signal/noise ratios of the analyte peak are 10 and 3, respectively. Each RSD and bias value should be no more than ±15% (±20% for LLOQ), which suggested an acceptable range. The data demonstrated that this LC−MS/MS method is good for the quantitative analysis of lamivudine in rat plasma. These outcomes demonstrated that the method was appropriate for pharmacokinetics studied on lamivudine in animal models.

Different lamivudine plasma concentrations of 5, 50, and 500 ng/mL were used to assess the matrix effects and recovery. The average recovery and matrix effect values for lamivudine in biological samples were 105.7−119.6 and 88.9−94.8%, respectively, and these values are presented in Table 2. The matrix effect data suggested that ion amplification occurred. The results showed no significant differences within the analytical range. After comparing the peak responses of the postextraction and spiked samples, it was clear that the ratios of the peak responses were within acceptable limits. The high reproduc-

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**Table 1. Intra- and Interassay Precision (% RSD) and Accuracy (% Bias) for the Determination of Lamivudine in Rat Plasma**

| nominal concentration (ng/mL) | observed concentration (ng/mL) | precision RSD (%) | accuracy bias (%) |
|------------------------------|---------------------------------|-------------------|------------------|
| Intra-Assay                  |                                 |                   |                  |
| 5                            | 5.72 ± 0.21                     | 3.67              | 14.41            |
| 10                           | 10.86 ± 0.48                    | 4.42              | 8.60             |
| 50                           | 53.27 ± 3.44                    | 6.46              | 6.55             |
| 100                          | 104.11 ± 3.46                   | 3.32              | 4.11             |
| 500                          | 509.12 ± 9.24                   | 1.81              | 1.82             |
| Interassay                   |                                 |                   |                  |
| 5                            | 5.40 ± 0.49                     | 9.07              | 7.94             |
| 10                           | 9.89 ± 0.25                     | 2.53              | −1.12            |
| 50                           | 50.57 ± 1.14                    | 2.25              | 1.14             |
| 100                          | 99.68 ± 0.69                    | 0.69              | −0.32            |
| 500                          | 499.16 ± 2.85                   | 0.57              | −0.17            |

*Data are expressed as the mean ± S.D. (n = 6).*
after three freeze-thaw cycles at ambient temperature, or after long-term storage for 1 month at −20 °C. The SPE method provided a recovery higher than 90%. Although the PPT method was used in a previous report, the SPE column materials are a hydrophilic N-vinylpyrrolidone and a lipophilic divinylbenzene copolymer, which readily retain the analyte. In addition, SPE is suitable for small sample volumes and consumes a small amount of solvent. Our results are consistent with a previous report that SPE provides high recovery, which may be due to the hydrophilicity of lamivudine.

2.3. Sample Preparation. To optimize the sample preparation procedure for rat plasma before UHPLC–MS/MS analysis, solid-phase extraction (SPE), liquid–liquid extraction (LLE), and protein precipitation (PPT) were examined. The recoveries were less than 40% by the LLE and PPT methods. The SPE method provided a recovery higher than 90%. Although the PPT method was used in a previous report, the SPE column materials are a hydrophilic N-vinylpyrrolidone and a lipophilic divinylbenzene copolymer, which readily retain the analyte. In addition, SPE is suitable for small sample volumes and consumes a small amount of solvent. Our results are consistent with a previous report that SPE provides high recovery, which may be due to the hydrophilicity of lamivudine.

2.4. Herbal–Drug Pharmacokinetic Interactions of S. chinensis Extract and Lamivudine. The mean lamivudine concentration versus time curves for rat plasma after the administration of lamivudine alone (10 mg/kg, i.v.) and pretreatment with oral S. chinensis (3 or 10 g/kg), with six rats in each group, are shown in Figure 3, and the pharmacokinetic results are presented in Table 4. The pharmacokinetic models of the one- or two-compartment model were compared with the Akaike information criterion (AIC). The lowest AIC values best represented the concentration versus time data. The data demonstrated that the AIC values for the one-compartmental model at treatment with only lamivudine (10 mg/kg) and pretreatment with oral S. chinensis (3 or 10 g/kg) were −15.13, −11.95, and −10.48, respectively, and the values with the two-compartmental model of the mean AIC values were −52.93, −44.19, and −35.15, respectively.

![Figure 3. Concentration–time curve of lamivudine in rat plasma after the administration of lamivudine alone (10 mg/kg, i.v.) and pretreatment with different oral doses of S. chinensis (3 g/kg; this dose is equivalent to a schizandrin dose of 5.2 mg/kg) and S. chinensis (10 g/kg; this dose is equivalent to a schizandrin dose of 17.3 mg/kg). Data are expressed as the mean ± S.D. (n = 6).](https://dx.doi.org/10.1021/acsomega.9b03922)

Table 2. Recovery and Matrix Effects of Lamivudine in Rat Plasma

| nominal concentration (ng/mL) | peak area | matrix effect (%) | recovery (%) |
|-------------------------------|-----------|-------------------|--------------|
|                               | set 1     | set 2             | set 3        |
| Lamivudine                     |           |                   |              |
| 5                             | 3105 ± 302| 3266 ± 92         | 2907 ± 196   | 105.7 ± 7.0  | 88.9 ± 3.5  |
| 50                            | 3260 ± 322| 4043 ± 2843       | 3618 ± 863   | 119.6 ± 10.9 | 93.4 ± 3.0  |
| 500                           | 352860 ± 3790| 450269 ± 14948   | 373785 ± 43865| 116.6 ± 3.1  | 94.8 ± 9.0  |
| average                       | 62710 ± 2132| 78349 ± 1766    | 75073 ± 699  | 112.3 ± 7.0  | 92.4 ± 5.1  |
| internal standard             | 62710 ± 2132| 78349 ± 1766    | 75073 ± 699  | 125.0 ± 1.6  | 95.8 ± 1.3  |

“The data are expressed as the mean ± S.D. (n = 3). Recovery (%): set 2/set 1. Matrix effect (%): set 3/set 2.

Table 3. Stability Data for Lamivudine in Rat Plasma

| nominal concentration (ng/mL) | short-term stability | post-preparative stability | freeze–thaw stability | long-term stability |
|-------------------------------|----------------------|-----------------------------|-----------------------|---------------------|
| Blood                         |                      |                             |                       |                     |
| 5                             | 4.56 ± 0.87          | 8.30 ± 5.98                 | −2.78 ± 3.00          | 7.23 ± 5.28         |
| 50                            | 2.17 ± 2.24          | −4.85 ± 3.46                | 2.65 ± 4.34           | 3.69 ± 2.97         |
| 500                           | 4.18 ± 1.22          | 4.13 ± 2.76                 | −1.87 ± 1.22          | 1.86 ± 1.88         |

“The data are expressed as the mean ± S.D. (n = 3).
Table 4. Pharmacokinetic Parameters of Lamivudine in Blood after the Administration of Lamivudine (10 mg/kg, i.v.) or Pretreatment with Oral S. chinensis (3 and 10 g/kg, p.o.)

| parameter          | lamivudine (10 mg/kg) | lamivudine (10 mg/kg) + S. chinensis (3 g/(kg day)) | lamivudine (10 mg/kg) + S. chinensis (10 g/(kg day)) |
|--------------------|-----------------------|-----------------------------------------------------|----------------------------------------------------|
| one-compartment AIC| −15.13                | −11.95                                              | −10.48                                             |
| two-compartment AIC| −52.93                | −44.19                                              | −35.15                                             |
| C_{max} (µg/mL)   | 7.02 ± 2.37           | 7.83 ± 1.42                                         | 8.09 ± 2.39                                        |
| AUC (min/(µg mL)) | 74.53 ± 11.30         | 87.10 ± 3.51                                        | 78.74 ± 4.79                                       |
| t_{1/2} (min)     | 4.54 ± 1.16           | 4.31 ± 1.02                                         | 3.29 ± 1.10                                        |
| t_{1/2} (min)     | 27.40 ± 2.60          | 30.62 ± 5.43                                        | 21.49 ± 2.96                                       |
| CL (mL/(min kg))  | 136.8 ± 21.49         | 115.0 ± 4.66                                        | 127.4 ± 8.00                                       |
| MRT (min)         | 23.01 ± 4.33          | 26.98 ± 4.55                                        | 21.65 ± 2.95                                       |
| V_{d} (mL/kg)     | 3.09 ± 0.47           | 3.11 ± 0.61                                         | 2.77 ± 0.53                                        |

"Data are expressed as the mean ± S.D. (n = 6). Student’s t test was used to compare the differences between groups, and a value of P < 0.05 was considered statistically significant.

extract did not significantly alter the pharmacokinetics of lamivudine.

According to previous studies on drug metabolism, approximately 60% of clinically used drugs are related to cytochrome P450 (CYP).24,25 CYP3A4, CYP1A2, and CYP2E1 are the primary enzyme subtypes in the CYP450 system. CYP450 is the primary metabolic enzyme in the human liver and intestine.25,26 The presence of inhibitors of the CYP superfamily in herbs may cause botanical–drug interactions and lead to serious side effects. Therefore, this is a key clinical factor in the pharmacokinetics of potential herb–drug interactions.27,28 Our pharmacokinetic results demonstrated that the herb–drug coadministration of S. chinensis and lamivudine slightly increased lamivudine C_{max} at the two tested dosage levels, but the increase was not significant. The major route of oral lamivudine is unchanged by the kidneys and excreted in urine (approximately 70%), and approximately 5–10% is converted by hepatic metabolism into a trans-sulfoxide metabolite.29 Lamivudine is not significantly metabolized by cytochrome P450 enzymes; nor does it inhibit or induce this enzyme system. Previous studies have shown that S. chinensis extract had a significant inhibitory effect on the activities of rat CYP1A2, 2C6, 2C11, 2D2, and 2E1, and 3A1/2 in vitro. S. chinensis has been found to affect the pharmacokinetics of several drugs.30,31 In addition, previous studies have shown that some components of traditional Chinese medicine extracts can inhibit or induce the activity of members of the CYP superfamily, such as CYP 2E1, 1A2, and 3A4,32,33 which may cause significant pharmacological interactions.

In our study, by applying our improved methods for the detection of lamivudine, we found that pretreatment with low and high doses of S. chinensis did not significantly influence the pharmacokinetics of lamivudine. Despite the increased C_{max} and area under the curve (AUC) of lamivudine, significant differences in lamivudine pharmacokinetics after S. chinensis consumption were not observed. A possible reason for the lack of interaction is that most of the components of lamivudine are excreted through the kidneys and are not significantly metabolized by cytochrome P450 enzymes. The dose of S. chinensis does not affect CYP expression. Therefore, the possibility of drug interactions is low and there is also no significant difference in pharmacokinetics. Because different components of the herb may have their own biological characteristics, several factors, such as the dosage regimen or the herbal formulation, may affect the clinical applications.

3. CONCLUSIONS

In summary, a UHPLC–MS/MS analytical method was developed and precisely validated for the analysis of lamivudine in rat plasma. This method also provides useful preclinical pharmacokinetic information that, based on the above study, shows that S. chinensis and lamivudine may not have significant interactions. This study provides safety information regarding the lack of significant herb–drug pharmacokinetic interactions and should be very important as a reference for additional clinical applications.

4. MATERIALS AND METHODS

4.1 Reagents and Chemicals. Urethane, poly(ethylene glycol) (PEG 400), and methyl yellow were purchased from Sigma-Aldrich (St. Louis, MO). Lamivudine was obtained from the United States Pharmacopeia (USP, Rockville, MD). S. chinensis was obtained from Chia-Hui Inc., Taipei, Taiwan (product lot no. Q0606). LC–MS grade methanol and formic...
acid were purchased from E. Merck (Darmstadt, Germany). Triply deionized water was used in these experiments.

4.2. Preparation of the S. chinensis Extract. The extraction was performed as follows: (1) the dried fruits of S. chinensis (1.8 kg) were soaked in 50% ethanol (24 L) in a stock pot for 120 min; (2) the extracted liquid was condensed by rotary evaporation to remove the solvent; and (3) water was removed using a freeze dryer. The resulting S. chinensis extract was stored at −80 °C. The extraction yield from the herb was 330 g (18.3%). The active ingredient in S. chinensis extract, schizandrin, was quantified by analytical techniques. The LC–MS/MS results are shown in Figure 4. The analytical method was performed on a Purostar STAR RP-18 end-capped (100 mm × 2.1 mm, 2 μm, Merck KGaA, Darmstadt, Germany) and the column oven was maintained at 40 °C. The mobile phase consisted of methanol/0.1% aqueous formic acid (85:15, v/v) at a flow rate of 0.2 mL/min, and the injection volume was 10 μL.

4.3. Experimental Animals. All experimental procedures were certified by the Institutional Animal Care and Use Committee of National Yang-Ming University (IACUC number: 1060813r). Urethane (1 g/kg) was used to anesthetize the rat. The Institutional Animal Care and Use Committee of National Yang-Ming University (IACUC administered at 7.5 g per dose34 for adult patients. The blood samples were collected via the tail artery. A 150 μL aliquot of rat blood was withdrawn from the rat tail artery after the animal experiment. Finally, lamivudine (10 mg/kg, i.v.) was injected into the femoral vein.

4.4. Sample Preparation of Lamivudine. A solid-phase extraction (SPE) method was applied to extract lamivudine from biological samples. First, Oasis HLB extraction cartridges (Waters Corp., Milford, MA) were equilibrated using methanol (2 mL) and triple deionized water (2 mL). Then, 50 μL of a real sample was added to the tube. Then, the SPE tube was washed using triple deionized water (1 mL) and 5% methanol. Finally, the lamivudine was eluted from the tube using acetonitrile. The eluent was collected in 2 mL microcentrifuge tubes and heated at 50 °C for 50 min to evaporate the solvent. The dried samples were redissolved in 50% methanol (190 μL) and an internal standard (10 μL), and then injected into the analytical equipment.

4.5. LC–MS/MS. The liquid chromatography–tandem mass spectrometry (LC–MS/MS) system consisted of a quaternary pump, an autosampler, and a column oven (Waters ACQUITY UHPLC, Milford, MA). An electrospray ionization (ESI) source in the positive-ionization mode was used in the experiments. The separation was performed on an ACQUITY UHPLC BEH Phenyl analytical column (100 mm × 2.1 mm, 1.7 μm, Waters, Dublin, Ireland) and maintained at 40 °C with a column oven. The mobile phase consisted of methanol/0.1% aqueous formic acid (85:15, v/v) at a flow rate of 0.2 mL/min, and the injection volume was 10 μL. The apparatus conditions were optimized to the following: source temperature, 150 °C; desolvation gas, nitrogen; cone voltage, 24 V; capillary voltage, 3.2 kV; desolvation gas flow rate, 800 L/h; desolvation temperature 400 °C; and collision energy, 14 V.

4.6. Method Validation. The method was validated to confirm that it met the requirements for analytical methods indicated by the FDA guidelines.36 The method was validated in terms of precision, accuracy, recovery, matrix effects, and stability.

4.6.1. Evaluation of Linearity, Accuracy, and Precision. The calibration curve was prepared in the range from 5 to 500 ng/mL. The calibration curve was prepared in the range from 5 to 500 ng/mL. The coefficient of variation for each standard profile was at least 0.99. The intraday precision and accuracy were estimated by analyzing three different quality control (QC) samples (low, middle, and high concentrations) six times in a single day. In contrast, the interday precision and accuracy were assessed by analyzing the QC samples on six consecutive days. Accuracy refers to the closeness of the experimental value to the real value based on the following formula: (bias %) = [(C_{obs} – C_{nom})/C_{nom}] × 100. The precision is used to describe the reproducibility of the results as follows: relative standard deviation (RSD %) = [standard deviation (SD)/C_{nom}] × 100. Except for the bias and coefficient of variation of the lower limit of quantitation (LLOQ), which were not permitted to exceed ±20%, others were kept within ±15%.

4.6.2. Matrix Effects and Recovery. The matrix effects and recoveries of lamivudine in rat plasma samples were evaluated at low, middle, and high QC concentrations (5, 50, and 500 ng/mL, respectively). The internal standard concentration was 5 ng/mL. Three extraction methods (sets 1–3) were used to assess the matrix effects and recoveries in the validation of the analytical method.

Set 1: Lamivudine stock solutions: Aliquots (10 μL) of lamivudine solution (5, 50, and 500 ng/mL) and internal standards (10 μL) were mixed with 180 μL of methanol in microcentrifuge tubes. After mixing, the solutions were delivered to an autosampler and injected into the analytical apparatus.

Set 2: Lamivudine solutions spiked after extraction. The rat plasma was handled using SPE. First, the extraction cartridge (Oasis HLB 30 μm) was equilibrated using methanol (2 mL) and triple deionized water (2 mL). Then, 50 μL of the sample was added to the tube. The SPE tube was washed using triple deionized water (1 mL) and methanol (5%). Finally, the lamivudine was eluted using acetonitrile. The eluent was collected in microcentrifuge tubes, and 10 μL of lamivudine solution (5, 50, and 500 ng/mL) was added to these tubes. These mixtures were heated at 50 °C for 50 min to evaporate the solvent. The dried extract was redissolved in 50% methanol (190 μL), internal standard (10 μL) was added, and the mixture was injected into the analytical apparatus.
Set 3: Lamivudine liquids spiked pre-extraction. All rat plasma samples were processed using SPE. Blank plasma (50 μL) and 10 μL of lamivudine solution (5, 50, and 500 ng/mL) were placed in microcentrifuge tubes and then mixed before extraction. Then, the extraction cartridge (Oasis HLB 30 μm) was equilibrated with methanol (2 mL) and triple deionized water (2 mL). Then, 50 μL of the spiked samples was added to the tube. The SPE tube was washed using triple deionized water (1 mL) and methanol (5%). Finally, lamivudine was eluted using acetonitrile. The eluent was gathered in microcentrifuge tubes and heated at 50 °C for 50 min to evaporate the solvent. The dried extract was redissolved in 50% methanol (190 μL) and internal standard (10 μL). Then, 10 μL of this mixture was injected into the analytical system. The matrix effect (%) was estimated using the following formula: sets 2/1. Recovery (%) was estimated using the following formula: sets 3/2.

4.6.3. Stability. The following four conditions were used to evaluate the stability of lamivudine in rat plasma: long-term, short-term, post-preparative, and freeze-thaw conditions. Low, medium, and high concentrations of lamivudine (5, 50, and 500 ng/mL) were selected for assessing stability.

(1) Short-term stability was evaluated based on quality control samples and stored at ambient temperature for 8 h.
(2) Post-preparative stability was assessed from quality control samples stored in an autosampler at 10 °C for 8 h.
(3) Long-term stability was assessed from quality control samples stored at −20 °C for 30 days.
(4) Freeze–thaw stability was assessed from quality control samples maintained at −20 °C for 24 h and thawed under ambient conditions. The freeze–thaw cycle was repeated for a total of three cycles. The stability (%) was evaluated as follows: stability (%) = (Cobs/Cnom) × 100%.

4.7. Pharmacokinetic Analysis and Statistics. The pharmacokinetic program of WinNonLin Standard Edition software version 1.1 with noncompartment and compartment models was used to calculate the pharmacokinetic parameters for oral and intravenous administration. Statistical analyses were performed using SPSS version 24.0 (SPSS, Armonk, NY: IBM Corp.). Student’s t test was used to compare the differences between groups, and a value of P < 0.05 was considered statistically significant. The Akaike information criterion (AIC) was utilized to evaluate the compartment model used for data processing. The pharmacokinetic parameters are explained as follows: the area under the curve (AUC) for concentration–time is defined as the plot of concentration of lamivudine in plasma against time. Cmax is defined as the highest concentration of lamivudine in blood after administration, and the time to reach this concentration is Tmax. Clearance (CL) is defined as the ability of the body to discharge drugs and is given by the following formula: CL = Dose/AUC. The mean residence time (MRT) of a drug molecule is the average time that it remains in the body.

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The authors contributed to this work as follows: C.-L.L. performed the study, analyzed the data, and prepared the manuscript. C.-H.H. and T.-H.T. designed the experiments, edited the paper, and secured funding.

Notes
The authors declare no competing financial interest.

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