Preclinical Herb−Drug Pharmacokinetic Interaction of Panax ginseng Extract and Selegiline in Freely Moving Rats

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ABSTRACT: Selegiline, an inhibitor of monoamine oxidase B, is prescribed during the early stages of Parkinson’s disease. The nutritional herbal medicine Panax ginseng C.A. Meyer has been reported to show potential neuroprotective activity; however, the herb−drug pharmacokinetic interaction between selegiline and P. ginseng extract has not been characterized. Our hypothesis is that the ginseng extract and selegiline produce pharmacokinetic interactions at certain doses. To investigate this hypothesis, a validated ultraperformance liquid chromatography−tandem mass spectrometry (UPLC−MS/MS) method was developed to monitor selegiline in rat plasma. Experimental rats were divided into groups treated with selegiline alone (10 mg/kg, i.v.; 30 mg/kg, p.o.), with the low-dose ginseng extract (1 g/kg, p.o., for 5 consecutive days) or with the high-dose ginseng extract (3 g/kg, p.o., for 5 consecutive days). The pharmacokinetic results demonstrated that the oral bioavailability of selegiline alone was approximately 18%; however, when rats were pretreated with low and high doses of the ginseng extract, the bioavailability of selegiline was 7.2 and 29%, respectively. These results suggested that the ginseng extract may produce a biphasic pharmacokinetic phenomenon. In summary, ginseng alters the oral bioavailability of selegiline, and these observations might provide preclinical information concerning the pharmacokinetic interactions between selegiline and herbal supplements.

1. INTRODUCTION

Selegiline [1-(−)-deprenyl; (−)-R-N,Nα-dimethoxy-N-2-propynyl-phenethylamine hydrochloride], an irreversible monoamine oxidase subtype B (MAO-B) inhibitor, has been used as a monotherapy for the early treatment of Parkinson’s disease. Although levodopa is the standard drug for managing Parkinson’s disease, several side effects, including dyskinesia, hallucinations, hypotension, and sleep disorders, have been observed. The combination of levodopa with selegiline has been applied in the treatment of the advanced course of this disease to delay the need for levodopa therapy and decrease the dose frequency of levodopa required. MAO-I inhibitors are effective for treating psychiatric disorders, especially depressive disorders, because they inhibit the degradation by monoamine oxidase of transmitter amines, including dopamine, serotonin, epinephrine, and norepinephrine. Selegiline is readily absorbed because of its lipophilic and slightly basic features and is rapidly metabolized in humans through the liver cytochrome P450 system. Then, selegiline is converted to L-methamphetamine, of which L-amphetamine is a secondary metabolite that is excreted as L-desmethyl-selegiline in urine.

Panax ginseng C.A. Meyer was shown to alter the pharmacokinetics of certain prescription medications through cytochrome P450 enzymes. Thus, it is important to investigate the interactions between herbal supplements and selegiline.

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to evaluate the pharmacokinetic interactions between *P. ginseng* extract and selegiline.

There are few reports in PubMed of analytical methods for the determination of selegiline.\textsuperscript{15,16} Some reports were based on liquid chromatography–mass spectrometry (LC–MS) using positive electrospray ionization but did not provide LC chromatograms or mass spectra,\textsuperscript{17} whereas other studies evaluated selegiline and its metabolites in urine with SPE.\textsuperscript{18} Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is valuable because it is sensitive, rapid, and selective; however, few bioanalytical methods with this technique have been developed.\textsuperscript{18}

Because nutritional supplements derived from ginseng extract possess potential neuroprotective activity and as selegiline is used to reduce symptoms in the early stage of Parkinson’s disease, it is possible that ginseng and selegiline could be used together in therapies. Our hypothesis is that ginseng extract and selegiline may interact via specific pharmacokinetic mechanisms. To investigate this hypothesis, a validated method for monitoring selegiline in rat plasma with ultraperformance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) was developed, and the preclinical pharmacokinetic interaction of ginseng extract and selegiline was examined in this study.

## 2. RESULTS AND DISCUSSION

### 2.1. Optimization of UPLC–MS/MS Conditions.

A Waters Acquity UPLC system coupled with a Waters Xevo tandem quadrupole mass spectrometer with a positive ionization electrospray interface were used to generate the product ion transitions. Optimization was achieved with the software IntelliStart (Waters Acquity UPLC system in MassLynx 4.1) and was modified by testing the cone voltage over a range of 12–20 V. A cone voltage of 14 V produced the best fragmentation for both selegiline and the noscapine internal standard (IS). The monitored ion transitions were *m/z* 188.2 and 118.98 for selegiline and *m/z* 414.29 and 220.11 for noscapine (IS), and the mass spectra are shown in Figure 1.

The analytes were separated on a C18 reverse-phase column (100 mm × 2.1 mm); the retention time of selegiline was 2.7 min, and the retention time of noscapine was 2.38 min. Mobile phases of 0.1% formic acid, 10 mM ammonium bicarbonate, 10 mM ammonium acetate, and 10 mM ammonium formate were tested. The mobile phase of 10 mM ammonium formate resulted in the most intense chromatographic peaks and was therefore selected as the mobile phase. To eliminate the interfering peak from blank plasma, methanol was added to the injection volume was 10 μL, and the total run time was 4 min. The chromatographic conditions resulted in high sensitivity and peak intensity, and the peak shape is shown in Figure 2.

### 2.2. Optimization of UPLC–MS/MS Conditions.

The linear range for selegiline was 5–500 ng/mL, and the calibration curve was described by *y* = 0.0085x − 0.0214, with good linearity (*r*² > 0.999). The lower limit of quantification (LLOQ) was 5 ng/mL with a signal-to-noise ratio (S/N) > 3. The matrix effect and recovery in rat plasma were estimated at low (5 ng/mL), medium (50 ng/mL), and high (500 ng/mL) concentrations and ranged from 83.4 to 90.9 and 97.1 to 105.85%, respectively (Table 1). The evaluation of the matrix effect (set 2/set 1) indicated that the plasma-based interferences and UPLC–MS/MS interferences were low and acceptable. The high reproducibility of the recovery demonstrated that protein precipitation was a reliable approach for sample preparation and that the IS was suitable for use in this analysis method.

The precision (± RSD) and accuracy (± bias) were calculated from intraday and interday assays, which were conducted by spiking blank plasma with selegiline at concentrations of 5, 10, 50, 100, and 500 ng/mL. The precision and accuracy are summarized in Table 2. The intraday precision ranged from 1.99 to 5.72%, with an accuracy of −6.76 to 10.37%, whereas the interday precision ranged from 0.26 to 13.95%, with an accuracy of −4.05 to 20.17%. The mean bias (%) and RSD (%) for selegiline in rat plasma varied within ±15%, but the LLOQ varied within ±20%, which was still acceptable according to the US Food and Drug Administration (US FDA) bioanalytical method validation guidelines. All the validation parameters demonstrated that replicate measurements were dependent on the quantification of selegiline in the rat plasma samples.

### 2.3. Stability.

The stability of the analyte at low, medium, and high concentrations (5 ng/mL, 50 ng/mL, and 500 ng/mL) was examined in triplicate after exposure to different conditions, including autosampler handling, short-term storage, long-term storage, and freeze–thaw cycles, as shown in Table 3. No significant degradation of selegiline was observed in the autosampler or during short-term or long-term storage conditions, but degradation was observed under freeze–thaw cycle conditions. Repeated freezing and thawing should be avoided during sample preparation of selegiline and similar analytes.
2.4. Pharmacokinetic Evaluation. The pharmacokinetic herb–drug interaction of *P. ginseng* C.A. Meyer with selegiline following intravenous administration of one dose (10 mg/kg) of selegiline or following oral administration of one dose (30 mg/kg) of selegiline coadministered either with two doses of *P. ginseng* C.A. Meyer extract (1 and 3 g/kg for 5 consecutive days) or without ginseng extract was used to calculate the bioavailability of selegiline.

The mean concentration in rat plasma over time after the administration of selegiline is provided in Figure 3. The maximum plasma concentration (*C*\(_{\text{max}}\)) was 123.2 ng/mL in the selegiline-only group and 141.8 and 108.6 ng/mL in the coadministered ginseng extract group (1 and 3 g/kg/day, respectively; Table 4). There was no significant difference in *C*\(_{\text{max}}\) between the oral administration groups. The time required to reach *C*\(_{\text{max}}\) (*T*\(_{\text{max}}\)) was 5 min, indicating that selegiline was rapidly metabolized in the rats, similar to its metabolism in humans. The area under the curve (AUC) for the concentration–time relationship was 2.52 and 10.22 μg/mL for the coadministration group (1 and 3 g/kg/day, respectively) and 5.45 μg/mL for the selegiline-only group; the selegiline-only value was intermediate between the values for the coadministration groups. The clearance (CL) in the coadministration group was opposite that in the selegiline-only group. The CL was lower in the 3 g/kg/day ginseng coadministration group (3.72 L/min/kg) than in the 1 g/kg/day ginseng group (12.47 L/min/kg). As a result, the AUC was higher in the 3 g/kg/day ginseng group than in the 1 g/kg/day ginseng group. After pretreatment with ginseng extract at 3 g/kg, a decreased CL rate, an increased half-life (*t*\(_{1/2}\)), and an increased mean residence time (MRT) were observed; however, the group coadministered 1 g/kg ginseng extract appeared to show stable trends characterized by significant decreases in the above parameters.

We investigated the major metabolic pathways of selegiline and the effects of *P. ginseng* extracts on CYP450 in rats and humans in a previously published paper.\(^{19,20}\) Cytochrome P450 enzymes, including CYP1A2, CYP3A4, and CYP2B6, participate in the metabolism of selegiline, which forms

| nominal concentration (ng/mL) | observed concentration (ng/mL) | precision RSD (%) | accuracy Bias (%) |
|-----------------------------|-----------------------------|------------------|------------------|
| **Intra-Assay**              |                            |                  |                  |
| 5                           | 5.2 ± 0.15                  | 2.92             | 4.06             |
| 10                          | 11.04 ± 0.63                | 5.68             | 10.37            |
| 50                          | 46.64 ± 2.67                | 5.72             | -6.76            |
| 100                         | 104.1 ± 2.07                | 1.99             | 4.1              |
| 500                         | 510.39 ± 14.3               | 2.80             | 2.08             |
| **Inter-Assay**             |                            |                  |                  |
| 5                           | 6.01 ± 0.84                 | 13.95            | 20.17            |
| 10                          | 10.62 ± 0.66                | 6.17             | 6.18             |
| 50                          | 47.97 ± 0.88                | 1.84             | -4.05            |
| 100                         | 100.3 ± 2.2                 | 2.19             | 0.3              |
| 500                         | 500.3 ± 1.31                | 0.26             | 0.06             |

\^Data are expressed as the mean ± SD (\(n = 6\)). RSD % = [SD/observed concentration (\(C_{\text{obs}}\))] × 100%. Bias % = 100 × [\(C_{\text{obs}}\)−nominal concentration (\(C_{\text{nom}}\))] / \(C_{\text{nom}}\).
CYP3A4 (minor), as explained in Figure 4. Alteration of selegiline metabolism consists of CYP1A2 (major) and majority of the drug-metabolizing CYP family involved in other research, suggesting that selegiline. In the low-dosage (1 g/kg/day) ginseng group, isoforms, which may be consistent with our the activity of CYP1A2 but reduced the activity of CYP3A, whereas CYP1A2 participates in the formation of desmethyl-selegiline. CYP2B6 showed higher activity in rats was observed in P. ginseng and selegiline. Both CYP3A4 and CYP2B6 are involved in two major oxidative pathways, whereas CYP1A2 participates in the formation of desmethyl-selegiline only. CYP2B6 showed higher affinity for the metabolism of selegiline than CYP1A2 and CYP3A4 in humans. However, the amount and activity of CYP450 in different species should be taken into consideration. In rats, the majority of the drug-metabolizing CYP family involved in selegiline metabolism consists of CYP1A2 (major) and CYP3A4 (minor), as explained in Figure 4. Alteration of the activity of CYP450 by P. ginseng in rats was observed in other research, suggesting that P. ginseng significantly enhanced the activity of CYP1A2 but reduced the activity of CYP3A isoforms, which may be consistent with our findings about the pharmacokinetic herb–drug interaction of P. ginseng and selegiline. In the low-dosage (1 g/kg/day) ginseng group, which showed increased CYP1A2 activity, selegiline degradation resulted in the formation of desmethyl-selegiline. However, the high-dosage (3 g/kg/day) ginseng group showed inhibition of CYP3A4 in two major oxidative pathways, which prevented selegiline degradation.

3. CONCLUSIONS

A validated UPLC–MS/MS analytical method was established for quantifying selegiline in rat plasma and successfully applied to determine the herb–drug pharmacokinetic interactions of P. ginseng extract with selegiline. The results indicated that during the administration of P. ginseng extract (1 g/kg/day for 5 days), significant decreases in the AUC and t1/2 for selegiline were observed as well as an increase in the CL rate, which provides important clinical information for the prescription of ginseng and selegiline.

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents. Selegiline, noscapine, urethane, heparin sodium, and ammonium formate were obtained from Sigma-Aldrich Chemicals (St. Louis, MO). LC–MS-grade methanol was purchased from J.T. Baker, Inc. (Phillipsburg, NJ). Triple-deionized water (Millipore, Bedford, MA) was used for UPLC–MS/MS analysis. Selegiline was dissolved in methanol to prepare the standard solution (1 mg/mL) and was diluted by methanol to generate the gradient series.

4.2. UPLC–MS/MS Conditions. A Waters Acquity UPLC system combined with a Purospher STAR RP-18 end-capped column (100 mm × 2.1 mm, 2 μm, Merck KGaA, Darmstadt, Germany), which was maintained in a column oven at approximately 40 °C, was used for all analyses. The UPLC system was equipped with a Waters Xevo tandem quadrupole mass spectrometer operated in positive electrospray ionization mode. All parent ion transitions, product ion transitions, cone voltages, and collision energies were optimized and modified with the MassLynx 4.1 software data platform (Waters Acquity).

Table 3. Stability of Selegiline in Rat Plasma

| nominal concentration (ng/mL) | autosampler stability (%) | short-term stability (%) | long-term stability (%) | freeze–thaw stability (%) |
|-------------------------------|---------------------------|--------------------------|-------------------------|---------------------------|
| 5                             | 89.83 ± 3.86              | 90.43 ± 2.57             | 88.63 ± 2.32            | 66.36 ± 1.79              |
| 50                            | 100.1 ± 6.97              | 91.19 ± 7.33             | 87.75 ± 1.44            | 59.40 ± 0.68              |
| 500                           | 96.43 ± 8.44              | 83.43 ± 8.81             | 88.69 ± 3.41            | 67.27 ± 1.42              |

*Autosampler stability: the sample was placed in an autosampler at 10 °C for 6 h. Short-term stability: the sample was placed in plasma at 4 °C for 6 h. Long-term stability: the sample was placed in plasma at −20 °C for 2 weeks. Freeze–thaw stability: the sample was placed in plasma at −20 °C for 12 h and then at room temperature for 12 h, and this cycle was repeated three times. Formulation = (peak area of processed sample/peak area of freshly prepared sample) × 100.

Table 4. Pharmacokinetic Parameters of Selegiline in Rats

| parameter                     | selegiline (10 mg/kg, i.v.) | selegiline (30 mg/kg, p.o.) | selegiline (30 mg/kg, p.o.) + P. ginseng (1 g/kg/day) | Selegiline (30 mg/kg, p.o.) + P. ginseng (3 g/kg/day) |
|-------------------------------|-----------------------------|-----------------------------|--------------------------------------------------------|--------------------------------------------------------|
| C0 (ng/mL)                    | 605.3 ± 213.8               | 123.2 ± 70.23               | 141.87 ± 41.03                                         | 108.6 ± 49.97                                         |
| Cmax (ng/mL)                  | 11.58 ± 1.742               | 5.46 ± 2.59                 | 2.32 ± 0.66                                            | 10.22 ± 5.27                                         |
| AUC (min μg/mL)               |                            |                            |                                                        |                                                        |
| t1/2 (min)                    | 110.3 ± 26.63               | 139.8 ± 89.5                | 30.35 ± 10.56                                          | 267.03 ± 315.13                                       |
| CL (L/min/kg)                 | 0.87 ± 0.13                 | 6.59 ± 3.02                 | 12.47 ± 2.72                                          | 3.72 ± 2.19                                           |
| MRT (min)                     | 98.24 ± 39.3                | 180.8 ± 131.1               | 34.91 ± 11.05                                          | 368.8 ± 312.0                                         |
| bioavailability (%)           | 18%                         | 7.2%                        | 29%                                                    |                                                        |

*Data are expressed as the mean ± SD (n = 6). p < 0.05 compared with selegiline (30 mg/kg, p.o.) only group by Student’s t-test analysis in SigmaPlot. p < 0.01 compared with selegiline (30 mg/kg, p.o.) only group by Student’s t-test analysis in SigmaPlot.
Male Sprague-Dawley rats (6 weeks, 230 ± IN, USA) and water were freely available at all times. Laboratory rodent diet 5001 (PMI Feeds, Richmond, IN, USA) was administered to the experimental rats was increased to 30 mg/kg orally in this study.

P. ginseng extract was dissolved in triple-deionized water and administered by gastric gavage at doses of 1 g/kg and 3 g/kg for 5 consecutive days during the study. Each group contained six rats. On the fourth day, after 1 h of ginseng extract administration, a rat was anesthetized with pentobarbital sodium (50 mg/kg) by intraperitoneal administration, and a PE 50 tube was catheterized in the right jugular vein for blood sampling. The catheter was fixed on the dorsal neck region, bypassing the skin, and capped with a stopper. After surgery, the rats were allowed to recover in an experimental animal cage for 1 day before drug administration. On the fifth day, P. ginseng extract was administered 1 h before selegiline administration, and a 150 μL blood sample was collected from the right jugular vein at 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, and 240 min after selegiline (30 mg/kg, p.o.) administration. Blood samples were centrifuged at 6000 g for 10 min at 4 °C to obtain plasma. Plasma was stored at −20 °C until analysis.

P. ginseng (300 g) was cut into pieces and soaked in 50% ethanol (1 L) for 30 min. The solution was then continuously boiled for 20 min, and another 500 mL of 50% ethanol. This cycle was repeated three times. The resulting solution was centrifuged at 6000 g for 10 min, and the supernatant was filtered through 90 mm filter paper. A rotary evaporator was used to remove the ethanol solvent. Then, the extracts were freeze-dried to obtain powder for experimental use.

Figure 4. Majority of the drug-metabolizing CYP family involved in the metabolism of selegiline consists of the human CYP2B6 and rat CYP3A4 enzymes.

UPLC system, Milford, USA). Chromatographic separation was carried out with isocratic elution by a mobile phase consisting of 10 mM ammonium formate (pH 6.40)/methanol [20:80 (v/v)]. The total run time was 4 min, the injection volume was 10 μL, and the flow rate was set at 0.2 mL/min. The MS conditions were as follows: electrospray ionization, positive mode; capillary voltage, 2.9 kV; cone voltage, 14 V; collision energy, 12 V; desolvation temperature, 400 °C; source temperature, 150 °C; desolvation gas flow, 800 L/h; cone gas flow, 60 L/h; and collision gas, argon. The monitored ion transitions were m/z 188.2 and 118.98 for selegiline and m/z 141.29 and 220.11 for noscapine (IS).

4.3. Experimental Animals and Drug Administration. Male Sprague-Dawley rats (6 weeks, 230 ± 20 g) were procured from the National Yang-Ming University Animal Center, Taipei, Taiwan. All experimental procedures were certified by the Institutional Animal Care and Use Committee of National Yang-Ming University (IACUC no. 1070519) and were performed according to the National Research Council guidelines. Rats were fed and housed with a 12 h light/dark cycle. Laboratory rodent diet 5001 (PMI Feeds, Richmond, IN, USA) and water were freely available at all times.

4.3.1. Intravenous Administration of Selegiline. Urethane (1 g/kg) was used to anesthetize rats via intraperitoneal injection, and polyethylene tubing (PE50) was implanted at approximately 3–4 cm in both the right jugular vein for blood collection and the left femoral vein for drug administration. A 150 μL blood sample was collected at 5, 15, 30, 45, 60, 90, 120, 180, 240, and 360 min from the left jugular vein after selegiline (10 mg/kg, dissolved in normal saline, n = 6) was administered intravenously. Blood samples were placed in Eppendorf tubes containing heparin and centrifuged at 6000 g for 10 min at 4 °C to acquire plasma. Plasma was stored at −20 °C for analysis. The intravenous administration group was established to evaluate the bioavailability through the formulation \([\text{AUC}_{\text{p.o.}}/\text{dose}_{\text{p.o.}}]/(\text{AUC}_{\text{i.v.}}/\text{dose}_{\text{i.v.}})\] × 100.

4.3.2. Oral Administration of Selegiline. Selegiline hydrochloride capsules (5 mg) are intended for administration to patients. The maximum dose for humans is 10 mg per day, which was converted to the dose for rats (2 mg/kg). However, during the pilot study of this experiment, the content of selegiline in rat plasma was found to be far lower than the LLOQ of the UPLC–MS/MS method. Hence, the dose of dilutions at concentrations of 5, 10, 50, 100, and 500 ng/mL were processed with the protein precipitation sample preparation method. The ratio of the selegiline peak area to the noscapine (IS) peak area was calculated to verify that the calibration curve, matrix effect, recovery, accuracy, precision, and stability of the plasma spiked samples, were determined accordingly.

The calibration curve was obtained from a series of dilutions of the standard solution. After spiking blank rat plasma, a series of dilutions at concentrations of 5, 10, 50, 100, and 500 ng/mL were processed with the protein precipitation sample preparation method. The ratio of the selegiline peak area to the noscapine (IS) peak area was calculated to verify that the correlation coefficient \((r^2)\) was greater than 0.995.

The matrix effect and recovery were evaluated with three different sets of samples containing low (5 ng/mL), medium

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Supernatant was filtered through a 0.22 μm syringe filter for UPLC–MS/MS analysis.

Set 2. Blank plasma (50 μL) and methanol (150 μL) were vortexed for protein precipitation and centrifuged at 6000g for 10 min at 4 °C. A total of 180 μL of supernatant was transferred into an Eppendorf tube with the standard solution (10 μL) and IS (10 μL). After mixing, the solution was filtered through a 0.22 μm syringe filter for UPLC–MS/MS analysis.

Set 3. The standard solution (10 μL), IS (10 μL), blank plasma (50 μL), and methanol (130 μL) were vortexed for 5 min and centrifuged at 6000g for 10 min at 4 °C. The supernatant was filtered through a 0.22 μm filter prior to UPLC–MS/MS analysis.

The accuracy and precision were evaluated by analyzing six replicates on the same day (intraday assay) and on 6 consecutive days (interday assay). The precision [relative standard deviation (RSD %)] was defined as the average difference between individual measurements and was calculated as RSD % = [SD/Cnom] × 100%. The accuracy (bias %) refers to the difference between the observed value and the true value and was calculated as bias % = 100 × [(Cobs − Cnom)/Cnom]. The precision and accuracy values should be within ±15%, whereas the LLOQs should be within ±20%.

4.6. Stability. To test the stability of selegiline at low (5 ng/mL), medium (50 ng/mL), and high (500 ng/mL) concentrations within the linear range in rat plasma under different conditions, the stability was assessed under four conditions: autosampler storage, short-term storage, freeze–thaw cycling, and long-term storage.

To assess the autosampler stability, a processed sample was stored in an autosampler for 6 h at 10 °C. To assess short-term stability, the standard solution was placed in blank plasma at room temperature for 6 h and processed as described for the set 3 sample preparation. To assess freeze–thaw cycle stability, three freeze–thaw cycles were performed as follows: samples were frozen for 12 h at −20 °C and then thawed at room temperature for 12 h. To assess long-term stability, the standard solution was stored in blank plasma at −20 °C for a month and then processed. All of the processed samples were compared with freshly prepared samples, and the standard deviation for each condition was required to be within ±15%.

4.7. Pharmacokinetic Analysis. WinNonlin Standard Edition (Version 1.1, Scientific Consulting Inc., Apex, NC, USA) was employed to calculate the pharmacokinetic parameters. A noncompartment model was used to determine the pharmacokinetic parameters in this study. The main parameters included the AUC, Cmax, t1/2, CL, and MRT. The bioavailability was calculated as [(AUCp.o./dosep.o.)/(AUCi.v./dosei.v.)] × 100. SigmaPlot (version 12.0) was used to calculate the drug concentration versus time profiles and to perform the statistical analysis. Each pharmacokinetic parameter was further analyzed by Student’s t-test, and a p value < 0.05 was regarded as statistically significant. All data are presented as the mean ± SD (n = 6).

Author Contributions

L.Y. and C.-L.L. performed the study, analyzed the data, and prepared the paper. T.-H.T. designed the experiments, edited the paper, and secured funding.

Notes

The authors declare no competing financial interest.

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