Research Article

Pharmacokinetic and Bioavailability Studies of Galgravin after Oral and Intravenous Administration to Rats Using HPLC-MS/MS Method

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This paper presents a new high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method with a rapid analysis of 6 min to determine the concentration of galgravin in rat plasma so as to study its pharmacokinetic features and bioavailability in vivo. Schisandrin was selected as the internal standard (IS). After extracting the analyte from plasma samples with ethyl acetate, methanol-H₂O (0.1% formic acid) (85 : 15, v/v) was used as mobile phase to achieve chromatographic separation on a C18 reversed phase column. The MS detection was performed in positive ion mode using electrospray ionization (ESI) source. This method showed good linearity over the range of 1~500 ng/mL (R² > 0.999), and the lower limit of quantitation (LLOQ) was 1.0 ng/mL. The intraday precision and interday precision were both within 8.5%, whereas the accuracies were in the range of -2.6%~6.0%. The average recoveries of galgravin in rat plasma were between 92.3% and 99.3%. Moreover, galgravin was stable throughout storage and processing with all RSDs below 12.1%. After the successful application of this optimized method, the oral bioavailability of galgravin was determined to be 8.5%. This study will be helpful to the future research and development of galgravin.

1. Introduction

Lignans have attracted extensive attention of researchers owing to their widespread bioactivities including antioxidant, antitumor, and hepatoprotective effects, as well as antileishmanial and antimalarial activities in recent years [1–4]. Galgravin is a new tetrahydrofuran lignan, which has been found in some medicinal plants, such as Piper wallichii [5], leaves of Tridax procumbens [6], rhizomes of Acorus tatarinowii Schott [7], and the stems of Schisandra propinqua [8]. Modern pharmacological studies have found that galgravin can effectively inhibit platelet activating factor and possesses anti-inflammatory and analgesic activities [9, 10]. In addition, galgravin showed significant cardioprotective and neuroprotective activities [11, 12]. Galgravin also inhibits excessive bone resorption by inhibiting differentiation of osteoclast, which plays a significant role in bone protection [13]. Evidence suggests that the compound displays promising cytotoxic activity against HL-60 cells (human leukemia) in vitro and might become a potential anticancer drug [14].

Currently, there are few literature reports on galgravin. In addition to using 1H and 13C NMR to identify the structure of this compound, the researchers once used vacuum liquid chromatography, semipreparative HPLC system [10], and supercritical fluid chromatography [15] during the extraction and isolation of the active ingredients in which galgravin was included. The GC (gas chromatography) and HPLC were
also applied to determine the content of galgravin in the effective part of Chinese herbs [11, 16].

Although galgravin has a variety of pharmacological activities, there is no literature on its pharmacokinetics in vivo. It is widely known that HPLC-MS/MS method is much useful in the pharmacokinetic study of quantifying the main components of drugs because of its high selectivity and sensitivity. A new and efficient HPLC-MS/MS analysis method was established in this research and first successfully applied to investigate the pharmacokinetics and bioavailability of galgravin in two different ways of administration (intragastric and intravenous dosing). The study would provide some references for further research and application of this compound.

2. Materials and Methods

2.1. Reagents and Chemicals. Chromatographic grade methanol and acetonitrile were obtained from Thermo Fisher Scientific Co. Ltd. (Canada). Both ethyl acetate (Concord Technologies Ltd., Tianjin, China) and formic acid (ROE Co. Ltd., Newark, USA) were also of HPLC grade. Ultrapure water was prepared by a Milli-Q water purification system (Millipore, Milford, MA, USA). Galgravin (purity ≥ 98%) and schisandrin (purity ≥ 99%) were purchased from Chengdu Dexter Biotechnology Co. Ltd. The chemical structures of galgravin and IS are presented in Figure 1.

2.2. Chromatographic and Mass Spectrometry Conditions. The separation and detection of the analyte were carried out using the LC-MS/MS system, which comprises mainly of an Agilent-1200 high-performance liquid chromatography system (Agilent, USA) and an Agilent-6430 triple quadrupole tandem mass spectrometer (Agilent, USA).

The chromatographic separation was achieved by using a Waters XBridge™ BEH C18 column (2.5 μm, 4.6 × 50 mm) with column temperature of 25°C and a run time of 6 min. The mobile phase system was composed of methanol and 0.1% formic acid water (85:15, v/v) with column temperature of 25°C and a run time of 6 min. The mobile phase system was composed of methanol and 0.1% formic acid water (85:15, v/v). 5 μL sample was injected into HPLC-MS/MS system under the flow rate of 0.3 mL/min.

The positive ESI with multiple reaction monitoring (MRM) mode was applied for mass detection. The specific parameters of galgravin and IS are shown in Table 1, and other parameters were optimized and set as follows: atomizer pressure was 25 psi, capillary temperature was 320°C, capillary voltage was 4000 V, and flow rate of drying gas was 11 L/min.

2.3. Calibration Standard (CS) and Quality Control (QC) Sample Preparation. A certain amount of galgravin and IS reference material were weighed and dissolved with methanol to prepare respective stock solution at concentration of 1 mg/mL. Then, the stock solution of galgravin was diluted serially with methanol to prepare the working solutions over the concentration range of 5-2500 ng/mL. And IS working solution was at 200 ng/mL. All solutions were stored in a refrigerator at -20°C. Appropriate amount of working solution was added into blank rat plasma (100 μL) for preparation of serial CS solutions, yielding final concentrations of 1, 2, 5, 10, 25, 50, 100, 250, and 500 ng/mL for galgravin and 40 ng/mL for IS. The concentrations of QC samples for method validation were 2, 50, and 400 ng/mL, respectively.

2.4. Plasma Sample Preparation. After addition of 100 μL rat plasma, 20 μL methanol, and 20 μL IS working solution, the mixture was vortexed for 40 s. 1 mL ethyl acetate was then added into the centrifuge tube. It took 10 minutes to centrifuge the mixture under the condition of 12,000 × g and 10°C after vortex mixing of 3 min. The supernatant fraction was then transferred into another tube and evaporated to dryness under a gentle nitrogen stream. The obtained residue was redissolved with 100 μL methanol, followed by vortex for 3 min and centrifugation for 10 min at 12,000 × g. Lastly, 5 μL of the supernatant fluid was used to analyze with HPLC-MS/MS.

2.5. Method Validation

2.5.1. Specificity. The specificity of method was investigated by comparing chromatograms of blank plasma samples from 6 rats with blank plasma samples spiked with galgravin and IS and an experimental plasma sample from a rat at 1 h after gavage of galgravin.

2.5.2. Linearity and Sensitivity. The calibration curve was constructed at nine concentration levels (1-500 ng/mL for galgravin), and regression equation was calculated with the concentration of galgravin as independent variable (x), the peak area ratio of galgravin to IS as dependent variable (y), and 1/x as weight factor. Then, the correlation coefficient (R²) was used to evaluate the linearity. The sensitivity of the method was reflected by LLOQ with signal-to-noise ratio (SNR) greater than 10. At this concentration, not only should the relative error (RE) be within ±20% but also the relative standard deviation (RSD) was less than 20%.

2.5.3. Precision and Accuracy. QC samples at three concentrations (n = 6) were prepared and analyzed continuously for 3 days to investigate intra- and interday precision and accuracy. The precision and accuracy are expressed by RSD and RE, respectively. Meanwhile, the acceptance criteria were RSD ≤ 15% and RE within ±15%.

2.5.4. Extraction Recovery and Matrix Effect. The peak area ratio of the galgravin-spiked blank plasma sample to the analyte added in postextracted blank matrix was used to investigate the extraction recovery, and the peak area ratio of the postextraction matrix spiked with the analyte to the nonextracted QC standard solution injected directly was used to study the matrix effect.

2.5.5. Stability. In this study, the investigated stability items of QC samples (2, 50, and 400 ng/mL) were as follows: short-term stability (2 h at room temperature), freeze-thaw stability (three freeze-thaw cycles), postpreparative stability (12 h in autosampler), and long-term stability (1 week at -80°C).

2.6. Experimental Study on Pharmacokinetics. Twelve male Sprague-Dawley rats (250 ± 10 g, SPF grade) were randomly
The peak shape of the analyte obtained by CORTECS™ C18 column (2.7 μm, 2.1 × 50 mm) was asymmetric compared to X Bridge™ BEH C18 column (2.5 μm, 4.6 × 50 mm), and the chromatographic response significantly reduced using ZORBAX Eclipse C18 column (3.5 μm, 2.1 × 100 mm) with longer analysis time compared to X Bridge™ BEH C18 column (2.5 μm, 4.6 × 50 mm). Therefore, the X Bridge™ BEH C18 column (2.5 μm, 4.6 × 50 mm) was selected finally. Meanwhile, the influence of different mobile phase combinations (methanol-water, acetonitrile-water) or added buffers (phosphoric acid, formic acid) on the chromatographic behavior of the target compound was investigated. Methanol and 0.1% formic acid water were selected where the symmetric peak shape, appropriate retention time, and detection sensitivity. The optimal column temperature was 25°C. The major MS spectrometry parameters were optimized individually to obtain better responses. Results showed that the signal response of galgravin and IS in positive ion mode was higher in contrast to the negative mode. In addition, the optimal ion transition pairs for quantitation were at m/z 373.3 → 235.2 for galgravin and m/z 433.2 → 384.3 for IS in MRM mode.

3.2. Investigation of Sample Preparation Method. Three extraction methods were investigated to find an appropriate preparation method of plasma sample in this study. It was found that liquid-liquid extraction (LLE) with ethyl acetate provided better recovery and process efficiency of galgravin compared to protein precipitation with methanol and acetonitrile. Hence, LLE was selected to extract the analyte from biological samples.

3.3. Validation of Analytical Method

3.3.1. Specificity. The typical MRM spectra of blank plasma (a), blank plasma spiked with galgravin and IS (b), and plasma sample after gavage (c) are displayed in Figure 2. There was no obvious endogenous plasma matrix interference found at the retention time of galgravin (4.19 min) and IS (3.38 min).

3.3.2. Linearity and Sensitivity. Good linearity was attained in the range of 1-500 ng/mL. The typical regression equation of calibration curve was $y = 0.020756x + 0.005814$ ($R^2 > 0.999$).

Table 1: Mass spectrum parameters of galgravin and IS.

| Compound     | Parent ion (m/z) | Product ion (m/z) | Collision energy (V) | Fragmentor (V) | Ion mode |
|--------------|-----------------|------------------|----------------------|----------------|----------|
| Galgravin    | 373.3           | 235.2            | 114                  | 9              | Positive |
| Schisandrin  | 433.2           | 384.3            | 100                  | 15             | Positive |

Figure 1: The chemical structures of (a) galgravin and (b) IS.
pharmacokinetic parameters were calculated according to the trend of plasma concentration with time. The major intravenous administrations of galgravin. Figure 3 illustrates samples obtained from rats that received intragastric and method was proved to be successful to determine the plasma The novel analytical

3.4. Application of Analytical Method. Table 4 sums the stability data of QC samples that received intragastric and intravenous administrations of galgravin. Figure 3 illustrates the trend of plasma concentration with time. The major pharmacokinetic parameters were calculated according to the noncompartment model and presented in Table 5 in the form of mean ± standard deviation (SD).

The concentration of galgravin in plasma reached its peak \(C_{\text{max}}\), 48.42 ± 37.66 ng/mL at 2.08 h \((T_{\text{max}})\) and declined with the \(T_{1/2}\) of 3.99 h after a single oral administration to rats (20 mg/kg), suggesting that galgravin had a moderate speed of absorption and metabolism in blood circulatory system after oral administration. However, \(C_{\text{max,oral}}\) was much lower than \(C_{\text{max,v}}\), which means that the absorption of galgravin was not very well. The \(AUC_{(0-t)}\) \((314.25 ± 179.84 \text{ h ng/mL})\) was close to the \(AUC_{(0-\infty)}\) \((315.75 ± 179.70 \text{ h ng/mL})\), which indicated that the monitoring time of this study was reasonable. After intravenous injection of galgravin solution at 2 mg/kg, the \(C_{\text{max}}\) \((36.80 ± 63.59 \text{ ng/mL})\) was achieved at 0.03 h without absorption process; then, plasma concentration of galgravin decreased exponentially. Short MRT \((1.27 ± 0.19 \text{ h})\) and \(T_{1/2}\) \((1.34 ± 0.21 \text{ h})\) suggested fast elimination tendency of galgravin. The \(AUC_{(0-t)}\) and \(AUC_{(0-\infty)}\) were 369.56 ± 66.06 h ng/mL and 371.36 ± 66.59 h ng/mL, respectively. Finally, the calculated oral bioavailability of galgravin was 8.5%.

By comparison, intravenous administration

3.3.3. Precision and Accuracy. The results of intra- and interday precision and accuracy are shown in Table 2. All RSD values of QC samples did not exceed 8.5% and RE ranged from -2.6% to 6.0%. The results were within acceptable limits of precision and accuracy.

3.3.4. Extraction Recovery and Matrix Effect. As listed in Table 3, the mean extraction recovery of galgravin was 94.4%, 99.3%, and 92.3% at 2, 50, and 400 ng/mL, respectively. No significant signal suppression or enhancement was observed with this method. The data of matrix effect ranged from 83.1 ± 5.1% to 100.0 ± 6.1%.

3.3.5. Stability. Table 4 sums the stability data of QC samples under different conditions. The results suggested that the analyte has good stability with all RSDs ≤ 12.1%.

3.4. Application of Analytical Method. The novel analytical method was proved to be successful to determine the plasma samples obtained from rats that received intragastric and intravenous administrations of galgravin. Figure 3 illustrates the trend of plasma concentration with time. The major pharmacokinetic parameters were calculated according to

| Spiked conc. (ng/mL) | Intraday Measured conc. (ng/mL) | Intraday RSD (%) | Interday Measured conc. (ng/mL) | Interday RSD (%) |
|----------------------|---------------------------------|------------------|---------------------------------|------------------|
| 2                    | 2.10 ± 0.16                     | 5.0              | 2.12 ± 0.18                     | 6.0              |
| 50                   | 50.25 ± 0.89                    | 0.5              | 51.32 ± 3.14                    | 2.6              |
| 400                  | 401.08 ± 15.63                  | 0.3              | 389.68 ± 26.24                  | -2.6             |

| Spiked conc. (ng/mL) | Extraction recovery (%) | RSD (%) | Matrix effects (%) | RSD (%) |
|----------------------|-------------------------|---------|--------------------|---------|
| 2                    | 94.4 ± 3.6              | 3.8     | 100.0 ± 6.1        | 6.1     |
| 50                   | 99.3 ± 6.5              | 6.5     | 96.6 ± 6.3         | 6.5     |
| 400                  | 92.3 ± 5.7              | 6.2     | 83.1 ± 5.1         | 6.1     |

Figure 2: The typical MRM chromatograms of galgravin and IS: (a) blank plasma; (b) blank plasma spiked with galgravin and IS; (c) a rat plasma sample after oral administration of galgravin.
shows the advantage of a faster onset of action and would be beneficial for rapid clinical control of the condition.

4. Conclusion

In the study, an efficient and sensitive analysis method of HPLC-MS/MS was developed and optimized to determine galgravin in plasma after single-dose oral and intravenous administration to rats. The described method had been proved to be successful in studying the bioavailability of galgravin. The absolute bioavailability of 8.5% indicated oral malabsorption of galgravin. This paper provides the first pharmacokinetic and bioavailability study of galgravin, which might be helpful for its further research and applications.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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