Pharmacokinetics of atractylenolide I from *Atractylodes macrocephala* Koidz in rats by RP-HPLC

Cui-Qin Li, Lang-Chong He*, Yan-Jun Hu

School of Medicine, Xi’an Jiaotong University, Xi’an 710061, China

Abstract  **Aim** To establish an RP-HPLC method for the analysis of pharmacokinetics of atractylenolide I in rats.

**Methods** The biological samples were extracted with ethyl acetate. The chromatographic conditions were as follows: An ODS column (4.6 × 150 mm, I.D. 5 µm) was used. The mobile phase was acetonitrile/water (55:45) with a flow rate of 1.0 mL·min⁻¹ under the room temperature, and the detection wavelength was set at 220 nm.

**Results** The Lowest Limit of detection was 5.0 ng·mL⁻¹ (S/N>3). The linear range was 0.025 µg·mL⁻¹ ~ 2.5 µg·mL⁻¹ (r=0.9992) in rat plasma. The method recoveries for all samples were greater than 94% at the low, medium and high concentrations. The within-day and between-day precision were from 3.17% to 6.93% and 2.33% to 7.20%, respectively. After oral administration of atractylenolide I 80 mg·kg⁻¹, the concentration-time profiles of atractylenolide I fit a two compartment with the first absorption model. **T**<sub>max</sub> was (0.21 ± 0.04) h, and **C**<sub>max</sub> was (420 ± 35) ng·mL⁻¹, **AUC** was (1313 ± 146) (ng·h)·mL⁻¹.

**Conclusion** The method was accurate, stable and reliable, and can be used for the investigation of atractylenolide I in plasma of rats. The experimental results showed that atractylenolide I was easily absorbed, but its elimination was slow from 2 h to 24 h after oral administration.

**Keywords** Atractylenolide I; RP-HPLC; Pharmacokinetics.

Introduction

In traditional Chinese medicine, Bai Zhu (the rhizomes of *Atractylodes macrocephala* Koidz), a member of the **Compositae** can invigorate the spleen, and cure patients with splenic asthenia, anorexia, oedema, excessive perspiration and abnormal fetal movement [1]. The anti-inflammatory activity of *A. macrocephala* extract has been investigated by Yamahara [2]. Atractylenolide I is a sesquiterpenes compound isolated from *A. macrocephala*. [3] The compound fractionated from *A. japonica* rhizomes was found to be active compound in acetic acid-induced vascular permeability and chick embryo granuloma anti-inflammatory assays. [3] Atractylenolide I also could inhibit cotton-pellet granuloma formation. [5] When atractylenolide I was tested for inhibitory activity against 5-lipoxygenase (5-LOX) and cyclooxygenase (COX), it showed no remarkable effects against either of the enzymes [6]. But it was previously isolated as an antagonizing TLR4 component from *A. macrocephala*. [7]

Li wei et al [8-9] used HPLC to determine the atractylenolide I in medical material and processed product. However, the method of determining atractylenolide I in biological samples has not been reported in the literature. In this study, we have developed an RP-HPLC method for the determination of atractylenolide I in rats to study its pharmacokinetics. The procedure is sensitive, accurate and reproducible, and should be suitable for the support of this study. The pharmacokinetic parameters of atractylenolide I in rats have firstly been obtained.

**Materials and methods**

Reagents and chemicals
HPLC grade methanol and acetonitrile were obtained from Fisher Scientific (Pittsburgh, USA). Ethyl acetate, n-hexane, ether and petroleum ether were from Xi'an Analytical Reagent Factory (Xi'an, China). Silica G (ZEX-Ⅱ, 100 ~ 200 mesh) was from Qingdao Haiyang Chemical Co. Ltd (Qingdao, China). Other reagents used in the experiment were analytical grade and from commercial sources. *Atractylodes macrocephala* Koidz was from the TCM Store (Xi'an, China) and was ground to 60 mesh.

![Fig 1. Chemical structures of atractylenolide I and atractylenolide III](image)

**Apparatus and chromatographic conditions**

The chromatographic system consisted of a SHIMADZU LC-10ATVP chromatographic pump, SPD-10AVP and SPD-M10AVP detectors (SHIMADZU, Japan), a 7125 hand sampling valve (Rheodyne Company, USA), ANASTAR (AOTAI Technology Ltd., Tianjin, China) and CLASS-VP (SHIMADZU, Japan) chromatographic work stations.

The chromatographic conditions were as follows: An ODS column (4.6 × 150 mm, I.D., 5 µm) was used. The mobile phase was acetonitrile/water (55:45) with a flow rate of 1.0 mL·min⁻¹ under the room temperature, the detection wavelength was set at 220 nm, and the sample volume is 40 µL.

**Animals**

Male and female SD rats, weighing 225 ~ 275 g, were supplied by the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). All rats were maintained under standard conditions with normal access to food and water. 10 rats were randomly divided into two groups. The first group was used for preparation of blank plasma samples and the second for pharmacokinetic measurements. The rats were initially anesthetized with ether, and remained anesthetized throughout the surgery period. The part of femoral vein was exposed for plasma sample collection. The rats had recovered from anesthesia before the test.

**Preparation of atractylenolide I and atractylenolide III**

Air-dried and powdered *A. macrocephala* cut crude drugs (2 kg) were treated with supercritical CO₂ fluid (yield: 2.5%, w/w). A sample (50 g) of this crude extract, which was named BZC, was fractioned by silica gel chromatography with solvent systems of petroleum ether, petroleum ether - ether (1:1, v/v), ether and methanol to obtain four fractions, which were named BZC-1, BZC-2, BZC-3 and BZC-4, respectively. The yield of BZC-2 was 18 g (36%).

The BZC-2 fraction was subjected to column chromatography on C₁₈ silica gel eluted with methanol/water (67:33, v/v). The fractions were analyzed by HPLC. The chromatographic conditions were as follows: a Hypersil® ODS column (150 mm × 4.6 mm; I.D. 5 µm) was used. The mobile phase was methanol/water (67:33, v/v) with a flow rate of 1.0 mL·min⁻¹ under the room temperature, and the detection wavelength was set at 220 nm. Fractions containing identical compounds were pooled and the methanol was removed. The remaining aqueous solutions were extracted with ether, which was removed, leaving two white solid substances that were recrystallized repeatedly to yield two white needle crystals (compound A, yield: 1.1%, purity >99% by HPLC; compound B, yield: 1.7%, purity>99% by HPLC).

Compound A: m.p. 121~123 °C; IRmax(KBr)·cm⁻¹: 1750, 1680 (butenolide), 1648, 900 (double bond); MS m/z: 232(M⁺), 217, 204, 161, 147, 133, 122, 107; ¹H-NMR (CDCl₃, TMS internal
standard) δ (ppm): 0.90, 1.13, 1.33, 1.57, 1.60~1.66, 1.82, 1.87, 1.98, 2.31, 2.37, 2.38, 2.73, 4.61, 4.83 and 4.87; 13C-NMR (CDCl3, TMS internal standard) δ (ppm): 40.8, 22.3, 36.3, 148.4, 49.9, 25.7, 162.5, 77.9, 47.5, 36.9, 120.1, 174.6, 8.2, 16.4 and 106.9. The above data agreed with the data of atractylenolide I in literature report [3], thus the compound was confirmed as atractylenolide I (Fig 1).

Compound B: m.p. 166~169 ºC; IRmax(KBr)·cm−1: 3340, 1750, 1700, 1640, 900; MS m/z: 248(M+), 230, 220, 205, 191, 175, 159, 147, 133, 121, 105; 1H-NMR (CDCl3, TMS internal standard) δ (ppm): 1.03, 1.25, 1.56, 1.60~1.70, 1.81, 1.85, 1.96, 2.29, 2.40, 2.46, 2.62, 4.60 and 4.87; 13C-NMR (CDCl3, TMS internal standard) δ (ppm): 41.3, 22.3, 36.1, 148.6, 51.7, 24.7, 161.3, 103.9, 51.1, 36.7, 121.9, 172.9, 8.2, 16.6 and 106.8. The above data agreed with the data of atractylenolide III in literature report [3], thus the compound was confirmed as atractylenolide III (Fig 1).

Standard stock solutions
The standard stock solution was prepared by dissolving 10.00 mg of atractylenolide I in 10 mL methanol to yield a nominal concentration of 1.00 mg·mL−1. Atractylenolide III was as internal standard (I.S.) which concentration is 5.0 µg·mL−1. And the solutions were kept -20 ºC before use.

Preparation of assay standard samples
Atractylenolide I standard rat plasma samples (0.025, 0.050, 0.10, 0.25, 1.00 and 2.50 µg·mL−1) were prepared by spiking blank plasma with appropriate amounts of standard stock solution prepared above. Quality control samples to determine the recovery, accuracy and precision of the method were independently prepared by standard plasma solution at low (0.025 µg·mL−1), medium (0.10 µg·mL−1) and high (2.50 µg·mL−1) concentration. All samples were stored at -20 ºC until analysis.

Bio-samples collection
The drug-free rat plasma samples, obtained by femoral artery bleeding, were collected into heparinized tubes and stored at -20 ºC until use. A 1.0 mL saline solution of atractylenolide I was given to the rats of the second group at the dose of 80 mg·kg−1 by oral administration. 13 blood samples (0.5 mL each) were obtained from each rat at 0, 0.083, 0.167, 0.50, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12 and 24 h by femoral artery after administration of atractylenolide I. The blood samples were transferred to micro tubes (heparinized) and then centrifuged at 1000 g for 10 min. All rat plasma samples (0.2 mL each) were transferred to heparinized tubes and stored at -20 ºC until further analysis.

Extraction of bio-samples
A liquid-liquid extraction method was used for the extraction of atractylenolide I in bio-samples. To each 0.2 mL of plasma samples, after adding 10 µL of internal standard (5.0 µg·mL−1) solution, 1.0 mL of ethyl acetate was added. The mixture was vortex for 2 min. After centrifugation at 1000 g for 10 min, the upper organic layer was transferred to a clean tube. The procedure was repeated again. The organic solution was evaporated under a stream of nitrogen at 40 ºC. To the residue, 0.2 mL of acetonitrile/water (55:45) was added and centrifuged at 15000 g for 10 min. Aliquot (40 µL) of the supernatant was injected into the HPLC systems for analysis.

Statistical analysis
The pharmacokinetic parameters of atractylenolide I in rats were calculated by the 3p97 software supplied by The Mathematical Pharmacological Committee of the Chinese Pharmacological Society.

All data were expressed as means ± standard deviation. The statistical differences were estimated with student’s test.

Results and discussion
Chromatograms
The typical HPLC chromatograms obtained for atractylenolide I standard and I.S. are shown in Fig. 2. The retention time of atractylenolide I and I.S. was 9.9 min and 5.2 min. As shown in Fig. 2, the typical HPLC chromatograms obtained blank samples and bio-samples indicated that no interfering peaks were found in the bio-samples under the HPLC conditions above.
Fig 2. HPLC chromatograms of atractylenolide I in plasma samples

HPLC chromatographic conditions: An ODS column (4.6 × 150 mm, I.D, 5 µm) was used. The mobile phase was acetonitrile/water (55:45) with a flow rate of 1.0 mL·min⁻¹ under the room temperature, the detection wavelength was set at 220 nm, and the sample volume is 40 µL. (A: atractylenolide I and I.S.; B drug-free blood sample; C blood sample; 1 atractylenolide I; 2 I.S.)

**Linearly**

Linear calibration curve was obtained in the given concentration range of atractylenolide I in rat plasma sample. The response for atractylenolide I is linear over the concentration range of 0.025 µg·mL⁻¹ ~ 2.5 µg·mL⁻¹ for the plasma samples, with a correlation coefficient greater than 0.999. Standard curves were fitted to a first degree polynomial, \( y = 3.5721x - 0.0203 \), where \( y \) is the peak area ratio of atractylenolide I to atractylenolide III (I.S.), and \( x \) is atractylenolide I concentration (µg·mL⁻¹). The limit of detection (LOD) of the method was measured to be up to 5.0 ng·mL⁻¹ in plasma samples of atractylenolide I (S/N≥3). The method was sensitive enough to be used for the following studies.

**Precision and accuracy**

The precision and accuracy of the method were determined, and the data shown in Table 1 indicated that within-day precision was 3.17% ~ 6.93% and between-day precision was 2.33% ~ 7.20% for atractylenolide I, respectively. The method recoveries for all samples were more than 94%. The results showed that the method developed was suitable for the quantification of atractylenolide I over the concentration range selected.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (µg·mL⁻¹)</th>
<th>Within-day Found (µg·mL⁻¹)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>Between-day Found (µg·mL⁻¹)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>0.025</td>
<td>0.025</td>
<td>6.93</td>
<td>100.89</td>
<td>0.024</td>
<td>2.33</td>
<td>95.22</td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>0.099</td>
<td>3.68</td>
<td>98.27</td>
<td>0.096</td>
<td>3.53</td>
<td>96.36</td>
</tr>
<tr>
<td></td>
<td>2.500</td>
<td>2.348</td>
<td>3.17</td>
<td>94.19</td>
<td>2.501</td>
<td>7.20</td>
<td>99.37</td>
</tr>
</tbody>
</table>

**Stability**

Stability of samples was carried out over a period of 1 w at -20 °C by injecting same sample. The RSDs for the peak area of atractylenolide I measurements were 1.20% ~ 6.39% shown in Table 2. These results demonstrate that atractylenolide I samples are stable up to 1 w at -20 °C for the following studies.
Table 2. Summary of the stability of atractylenolide I in rat plasma samples

<table>
<thead>
<tr>
<th>Condition</th>
<th>Add (µg·mL⁻¹)</th>
<th>Found (µg·mL⁻¹)</th>
<th>RSD(%)</th>
<th>Recovers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20 °C for 1 week</td>
<td>0.025</td>
<td>0.023</td>
<td>1.20</td>
<td>92.17</td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>0.090</td>
<td>6.39</td>
<td>88.96</td>
</tr>
<tr>
<td></td>
<td>2.500</td>
<td>2.249</td>
<td>5.59</td>
<td>89.95</td>
</tr>
</tbody>
</table>

Fig 3. Mean plasma concentration-time curve of atractylenolide I in rats after oral administration of 80 mg·kg⁻¹ (n=5)

Pharmacokinetics

The blood concentration-time profile of atractylenolide I in rats after oral administration is shown in Fig 3.

Table 3. Pharmacokinetic parameters of atractylenolide I in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_max/h</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>C_max/(ng·mL⁻¹)</td>
<td>420 ± 35</td>
</tr>
<tr>
<td>T_1/2α/h</td>
<td>0.21 ± 0.10</td>
</tr>
<tr>
<td>T_1/2β/h</td>
<td>9.5 ± 1.9</td>
</tr>
<tr>
<td>T_1/2ka/h</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>V/F(μg·mL⁻¹)</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>AUC/(ng·h·mL⁻¹)</td>
<td>1313 ± 146</td>
</tr>
<tr>
<td>CLs/(mg·h⁻¹·µg·mL⁻¹)</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

The plot of atractylenolide I concentration versus time was relatively fitted to a two-compartment with the first absorption model by using 3p97 software. Then, the main pharmacokinetic parameters in this model were obtained and are listed in Table 3. The experimental results after 80 (mg·kg⁻¹)/day dose of atractylenolide I showed that T_max is (0.21 ± 0.04) h, C_max is (420 ± 35) ng·mL⁻¹, and AUC value is (1313 ± 146) (ng·h)·mL⁻¹. The parameters indicated that the absorption of atractylenolide I in rats was fast to reach maximal concentration within about 13 min. With an apparent volume of distribution (V/F) of (0.10 ± 0.02) L·kg⁻¹, the diffusion rate of atractylenolide I from blood to tissues was modicus. With a sum of clearance (CLs) of (0.06 ± 0.01) / (mg·h⁻¹)/(µg·mL⁻¹), however, the elimination rate varied greatly as shown in Fig 3. There is rapid elimination from 0.21 h to 2 h, and little slower from 2 h to 12 h. For this reason, it should be noted that there is a form of drug storage due to the binding of atractylenolide I to plasma protein.

Conclusions

A simple, sensitive and accurate RP-HPLC method has been developed for the determination of atractylenolide I in bio-samples. The method has been successfully applied to pharmacokinetic studies of atractylenolide I in rats. It is very helpful for a quantitative understand to the behavior of atractylenolide I from A. macrocephala in body.

References


*Correspondence to Prof Lang-Chong He, School of Medicine, Xi’an Jiaotong University, Xi’an 710061, China. tel: +86 029-82655392; fax: +86 029-82655451. E-mail: helc@mail.xjtu.edu.cn*