Pharmacokinetic study of eplerenone in rats after long-term coadministration with buckwheat tea

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Coadministration; Eplerenone; Hypertension; Pharmacokinetics; Tartary buckwheat

Abstract The aim of this study was to investigate the effect of long-term intake of Tartary buckwheat tea on the pharmacokinetics (PK) of eplerenone in rats. A validated high-performance liquid chromatography-mass spectrometry (HPLC-MS) method was established to determine the eplerenone in plasma, and the portal vein absorption model was applied to conduct the pharmacokinetic study. Two groups of animals—buckwheat tea group and control group—were involved in this study. Plasma samples were obtained at different time points after administration, and were separated on Shimadzu HPLC-MS 2020 instruments. The method showed good linearity (r = 0.9988) over a wide dynamic range (0.20–50 μg/mL). Within- and between-batch precisions ranged from 2.13% to 7.90%. The extraction recovery rates ranged from 91.96% to 94.96%. The data showed that in the Tartarian buckwheat group the area under the curve and maximum concentration of eplerenone were reduced compared with those of the blank group (p < 0.01), but the time to reach peak concentrations of eplerenone (p < 0.01) was prolonged. The results suggested that long-term consumption of Tartary buckwheat tea might induce the activities of the hepatic drug metabolizing enzyme, which can accelerate the metabolism of eplerenone. According to the results, the dosage of eplerenone should be adjusted in hypertension treatment trials when administered with Tartary buckwheat or Tartary buckwheat-containing dietary supplements to avoid potential drug interactions.

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Introduction

Tartary buckwheat, a dried mature seed of *Fagopyrum genera* in the Polygonaceae family, is mainly produced in southwestern and northern China. It is rich in flavonoids, oleic acid, vitamins, niacin, and dietary fiber [1,2]. The amount of flavonoids in Tartary buckwheat is 10–100 times higher than that of other buckwheat species, in which rutin makes up 70–90%.

Previous studies reported that flavonoids in Tartary buckwheat have hypoglycemic, serum lipid-lowering, and antihypertensive effects [3,4]. As a result, Tartary buckwheat tea has become a popular healthy drink in recent years. It is reported that the main bioactive ingredients of Tartary buckwheat—rutin, quercetin, kaempferol, and naringin [5,6], are inhibitors or activators of CYP3A4, CYP1A1, and CYP1A2 of the cytochrome P450 (CYP450) system [7–10]. Eplerenone, a type of a selective aldosterone receptor blocker, is mainly metabolized by CYP3A4 and has been used for the treatment of hypertension for a long time [11,12]. In clinics, rutin and quercetin frequently result in the emergence of drug–drug interactions by affecting the activities of drug-metabolizing enzymes [13–15]. If eplerenone is simultaneously coadministered with buckwheat tea to patients, the bioavailability and metabolism of eplerenone can be affected.

The purpose of this study is to investigate whether the long-term administration of buckwheat tea could affect the pharmacokinetic (PK) profile of eplerenone in rats. A validated high-performance liquid chromatography-mass spectrometry (HPLC-MS) method was established to determine the eplerenone in plasma, and the portal vein absorption model was applied to conduct the pharmacokinetic study. Two groups of animals, the buckwheat tea group and the control group, were used in the study. The result of this study was expected to provide an experimental foundation for the coadministration of eplerenone and buckwheat tea in the clinical treatment of hypertension.

Materials and methods

Chemicals and reagents

Tartary buckwheat tea was purchased from Zhengzhong Food Co., Ltd (Xichang, China). Eplerenone, rutin, and quercetin, were purchased from the National Institutes for Food and Drug Control (Beijing, China). Astragaloside (IS) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). β-Cyclodextrin, sodium phosphate buffer solution, and sodium chloride were purchased from Beijing Chemical Corporation (Beijing, China). HPLC-grade acetonitrile and methanol were obtained from Fisher Scientific (Iowa, USA). HPLC-grade formic acid was purchased from Kelong Chemical Industry (Chengdu, China), and ultrapure water was produced using a Millipore Milli-Q system (Millipore, Billerica, MA, USA). All the other reagents used were of reagent grade. The chemical structures of eplerenone and IS are shown in Figure 1.

Animal

Male Sprague–Dawley rats of specified pathogen free (SPF) grade were obtained from the Laboratory Animal Center of Sichuan Health Science Academy (Certificate No. SCXK 2004-6). The rats were housed in an animal room with air conditioning (22–24°C) and free access to food and water. Treatment of all animals was in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals. The experiments were carried out under the approval of Experiment Administration Committee of Sichuan Health Science Academy.

Drug administration

The Tartary buckwheat water solution for the experiment was prepared by boiling 17.8 g Tartarian buckwheat with 10 times of water for 5 minutes, followed by another 10-minute boiling cycle with 5 times of water. The solution was combined and concentrated to 100 mL. After centrifugation at 13,500g for 5 minutes, the clear supernatant was filtered by 0.22-μm (pore size) membrane. The filtrate was considered Tartarian buckwheat test solution, with a concentration of 0.178 g/mL.

We also prepared a lower concentration of Tartarian buckwheat solution as a substitute for the drinking water of the animals. Specifically, 5.34 g Tartarian buckwheat was treated under two boiling cycles (40 times of water for 5 minutes and 45 times of water for 10 minutes). The combined solution was concentrated to 300 mL, with a concentration of 0.0178 g/mL.

Eplerenone physico liquid was prepared by dissolving 1 g of eplerenone in 100 mL of a mixed solution that consisted of 15% β-cyclodextrin, 0.07 mol/L sodium phosphate buffer solution (pH 7.4), and 0.5% sodium chloride.

Animal experiment and drug administration

After 1 week of acclimatization, 20 male Sprague–Dawley rats (200–220 g) were randomly divided into the blank group and the Tartary buckwheat group (10 rats in each group). Rats in the Tartary buckwheat group were administered with Tartarian buckwheat water solution [1.78 g/kg, intragastric gavage (i.g.)] twice a day for 29 days. During these days, the lower concentration buckwheat solution substituted for the animals’ drinking water. Rats were fasted for 12 hours prior to the experiment with drinking water supply as usual. Rats in the two groups were orally administered with eplerenone solution (100 mg/kg). The blood samples (0.2 mL) were obtained 15 minutes, 30 minutes, 1 hour, 1.5 hours, 2 hours, 4 hours, 6 hours, 8 hours, and 10 hours after the administration. The blood samples were preserved in a 37°C water bath for 20 minutes and then centrifuged at 3500 rpm for 10 minutes. The upper phase was transferred and preserved in a refrigerator (at –80°C) for future analysis.

Sample preparation

Rat plasma sample (20 μL) was transferred into 4-mL tubes prior to adding 20 μL IS. The mixture was vortexed for 10 seconds, and acetonitrile (2 mL) was then added to form an emulsion. The emulsion was vortexed for 3 minutes followed by centrifugation at 4000 rpm for 8 minutes. The...
upper organic phase was transferred into a new tube and centrifuged at 13,400 rpm for 3 minutes. The upper phase was transferred into an injection vial for HPLC-electrospray ionization (ESI)-MS analysis.

Quantitative analysis of Tartarian buckwheat water solution

Quantitative analysis was carried out on a 1260 Agilent HPLC system. Chromatography separation was performed on an Agilent Zorbax SB-C18 guard column (12.5 mm × 4.6 mm inner diameter (i.d.), 5 µm). The multi wavelength detector (MWD) was operated at 375 nm, and the column temperature was set at 30°C. Then, 20 µL Tartarian buckwheat water solution was eluted at a flow rate of 0.8 mL/min, using a mobile phase of 0.2% (v/v) phosphoric acid in water and acetonitrile. The proportion of acetonitrile in the mobile phase was optimized as follows: 0–8 minutes, 28%; 8–18 minutes, 50%; 18–30 minutes, 100%; 30–35 minutes, 100%; 35–38 minutes, 28%.

Quantitative and qualitative analysis of plasma samples

Chromatography separation was performed on an Agilent Poroshell 120 EC-C18 column (2.7 µm, 4.6 × 100 mm) using a Shimadzu HPLC-MS 2020 system (Shimadzu MS Technologies, Japan), which was equipped with a binary solvent delivery system, an online vacuum degasser, an autosampler, an ultraviolet detector, a column oven, and labsolution 5.5 chromatographic work station. The column was maintained at 30°C and eluted at a flow rate of 1.0 mL/min, using a mobile phase consisting of 10 mmol/L ammonium acetate in water and acetonitrile. The proportion of acetonitrile in the mobile phase was 70%.

Mass spectrometry was carried out on a Shimadzu 2020 mass spectrometer with an ESI interface operating in positive ion mode.

Stock solutions, calibration standard, and quality control samples

Stock solutions of rutin and quercetin were prepared at 350 µg/mL in methanol. A stock solution of eplerenone was prepared at 500 µg/mL in methanol. The IS solution was prepared at 53.10 µg/mL in methanol. Calibration curves were prepared by spiking 20 µL of an appropriate working solution into 1.5-mL tubes, evaporating to dryness under a nitrogen stream, and spiking with 100 µL of blank rat plasma. The effective concentrations were 0.20, 0.50, 2, 5, 10, 20, and 500 µg/mL of eplerenone. Quality control (QC) samples were prepared in pool as a single batch for each concentration at concentrations of 0.20, 10, and 50 µg/L for eplerenone, and then divided into aliquots and stored in the freezer at −20°C. The IS working solution of 5.31 µg/mL was diluted from stock solution as needed. The rat plasma sample (calibration standards and QCs) were treated following the sample processing procedure.

Data analysis

Kinetica 5.0.11 software (Thermo Electron Corporation, USA) was used to fit the model. Maple15 software (Maple Corporation, Japan) was used to perform statistical analysis. One-way analysis of variance was carried out to compare data interclass. The least significant difference (LSD) test was appropriate to equal variance and Tamhane’s T2 test was appropriate to heterogeneity of variance. A p value < 0.05 was considered significant.

Results

Method validation

The method for determination of eplerenone in rat plasma by HPLC-MS was validated to meet the guidelines of the
Specificity

IS was chosen as the internal standard for eplerenone quantitation because of the similar chromatographic behavior with eplerenone. The typical HPLC chromatograms of blank plasma spiked with eplerenone/IS, and a sample obtained from an experimental rat at 1.5 hours after an oral administration of eplerenone solution (100 mg/kg) are shown in Figure 2. Under the assay conditions, eplerenone and IS were completely separated, and no endogenous substances interfered with eplerenone and IS in the plasma samples prepared from six different rats (Figure 2).

Calibration curves and limit of detection

The standard curve, established by plotting peak area ratios (y) of eplerenone to IS against analyte concentrations (x), exhibited good linearity over the concentration range of 0.20–50 μg/mL. The regression equation for eplerenone was y = 1.5006x + 0.0850. The lower limit of quantitation (LLOQ) for eplerenone was defined as 0.10 μg/mL based on Signal/Noise (S/N) = 10, and the limit of detection was estimated as 0.02 μg/mL based on S/N = 3. According to our previous analysis, 0.20 μg/mL eplerenone is suitable for the pharmacokinetic study and was selected as the lowest concentration on the calibration curves.

Precision and accuracy

The intraday and interday precision and accuracy for eplerenone were evaluated by assaying three QC levels (0.20, 10, and 50 μg/mL) on 6 consecutive days, and five samples were prepared for every level. The results are presented in Table 1. For each QC level of eplerenone, the intraday precision was lower than 7.51%, and the accuracy was between 95.16% and 101.67%; the interday precision was lower than 7.51%, and the accuracy was between 96.67% and 97.83%. These data suggested that the method was accurate and precise for the analysis of eplerenone in rat plasma samples.

Recovery

The extraction recovery of eplerenone was determined at three QC levels. Recovery was calculated by comparing the analyte/IS peak area ratios of each analyte in plasma samples to those in the matrices by spiking the extracted analyte-free plasma samples prior to chromatography. Recovery was calculated using the following formula: recovery (%) = concentration found/concentration spiked × 100%. The recovery rates of eplerenone were 94.96 ± 6.65%, 91.96 ± 1.96%, and 93.29 ± 4.21% (n = 3) at concentrations of 0.20, 10, and 50 μg/mL, respectively (Table 2).

Stability

Stability was evaluated at three QC levels. The stability of standard solutions was tested at room temperature for 2 hours and upon refrigeration (4°C) for 30 days. The stability of analytes was examined by keeping eplerenone QC samples in the autosampler tray for 24 hours and in a freezer at −20°C for 30 days; the freeze–thaw stability was obtained over three freeze–thaw cycles, by thawing at room temperature for 2–3 hours and then refreezing at −20°C for 12–24 hours. For each concentration and each storage condition, six replicates were analyzed in one analytical batch. The concentration of analytes after each storage period was related to the initial concentration, which was determined when the samples were originally prepared and processed. Results are shown in Table 3. The results of the stability tests indicated that the standard solutions are stable at room temperature for 2 hours (relative standard deviation (RSD) < 7.98%) and upon refrigeration (4°C) for 30 days (RSD < 7.90%). The analytes were stable in plasma samples for 24 hours (RSD < 7.18%) and in a freezer at −20°C for 30 days (RSD < 6.04%). The freeze–thaw stability was obtained over three freeze–thaw cycles (RSD < 5.78%).

Quantitative analysis of Tartarian buckwheat water solution

Quantitative analyses of rutin, isoquercitrin, quercetin, and kaempferol were carried out. The typical chromatograms of the Tartarian buckwheat water solution are shown in Figure 3. The content of rutin, isoquercitrin, quercetin, and kaempferol was determined to be 9.05, 0.25, 0.71, and 0.04 mg/g, respectively.

Pharmacokinetic and PK parameters analysis

The method was applied to the quantification of eplerenone in rat plasma samples after i.g. administration. The mean concentration–time curves of eplerenone after the administration of 100 mg/kg (i.g.) to the control and buckwheat tea groups are shown in Figure 4; the main pharmacokinetic parameters were calculated and are shown in Table 4.

One-way analysis of variance was carried out for statistical analysis to compare the data of the two groups. According to the data shown in Table 4, the maximal concentration of eplerenone (Cmax) was observed at 33.36 ± 3.35 μg/mL in the buckwheat tea group and 43.62 ± 3.24 μg/mL in the control group. The time to reach peak concentrations of eplerenone (Tmax) was observed at 1.00 ± 0.00 hours in the buckwheat tea group and 0.50 ± 0.00 hour in the control group. The area under the curve of eplerenone (AUClast) was 50.31 ± 4.28 μg h/mL in the buckwheat tea group and 58.79 ± 8.03 μg h/mL in the control group. The mean retention time for eplerenone was 2.01 ± 0.07 hours in the buckwheat tea group and 2.17 ± 0.13 hours in the control group.
Figure 2. Typical HPLC-MS chromatograms. (A) Blank Sprague-Dawley rat plasma spiked with astragaloside (IS); (B) blank Sprague-Dawley rat plasma spiked with eplerenone; (C) blank Sprague-Dawley rat plasma spiked with eplerenone and IS; and (D) Sprague-Dawley rat plasma obtained from an experiment rat at 1.50 hours after an oral administration of eplerenone solution (100 mg/kg). Peak 1 = IS; Peak 2 = eplerenone. HPLC-MS = high-performance liquid chromatography-mass spectrometry.
Studies have demonstrated that eplerenone was well absorbed and rapidly metabolized after oral administration [16]. In our work, the value of $T_{\text{max}}$ was consistent with that found in some previous studies, but the $C_{\text{max}}$ of eplerenone was much higher. It is most likely that eplerenone was dissolved in the sulfobutyl-$\beta$-cyclodextrin solution, which can be a good package for the drug molecule, forming a noncovalent complexes and enhancing the stability and water solubility of eplerenone.

CYP450 is considered to be the most important drug-metabolizing enzyme system in the body. Various studies have demonstrated that quercetin is an inhibitor of CYP3A4 and that it inhibits CYP3A4 enzyme activity in a concentration-dependent manner [8,14]. However, some studies suggest that different components of flavonoids may exert different effects on the hepatic microsomal monooxygenase system according to the route and the duration of administration [17]. An earlier study showed that there was no significant difference in Phase I or Phase II enzyme activities when quercetin was given for 14 days at a concentration of 1%. In contrast, the liver weight, protein content of microsomal and cytoplasmic, and activity of cytochrome P450 were substantially increased when 0.25% flavone was given [18].

It has been known that 6$\beta$-hydroxy eplerenone and 21-hydroxy eplerenone are the major metabolites in vitro in both humans and dogs, and that the metabolism of eplerenone was primarily mediated by CYP3A4 in humans and CYP3A12 in dogs [11]. However, 6$\beta$-OH eplerenone was the major metabolite in rats, and eplerenone metabolism to 6$\beta$-OH eplerenone (EP) was mediated primarily by CYP3A [12]. The results from our study clearly demonstrated that long-term coadministration of Tartary buckwheat tea can significantly reduce the $C_{\text{max}}$ and AUC, extend the $T_{\text{max}}$, and accelerate the elimination of eplerenone. The reduced bioavailability of eplerenone as a result of its coadministration with Tartary buckwheat suggested that Tartary buckwheat may promote the first-pass metabolism of eplerenone. It is predicted that long-term consumption of Tartary buckwheat tea might induce the activities of the hepatic drug-metabolizing enzyme.

### Table 1: Accuracy and precision for the determination of eplerenone in plasma samples ($n = 6$).

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (µg/mL)</td>
<td>Precision (%)</td>
</tr>
<tr>
<td>0.20</td>
<td>0.20 ± 0.02</td>
<td>7.51</td>
</tr>
<tr>
<td>10.00</td>
<td>9.87 ± 0.21</td>
<td>2.13</td>
</tr>
<tr>
<td>50.00</td>
<td>47.58 ± 1.74</td>
<td>3.67</td>
</tr>
</tbody>
</table>

SD = standard deviation.

### Table 2: Recovery of eplerenone in plasma samples ($n = 3$).

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (%)</td>
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<tr>
<td>0.20</td>
<td>94.96 ± 6.65</td>
</tr>
<tr>
<td>10.00</td>
<td>91.96 ± 1.96</td>
</tr>
<tr>
<td>50.00</td>
<td>93.29 ± 4.21</td>
</tr>
</tbody>
</table>

RSD = relative standard deviation; SD = standard deviation.

### Discussion

Studies have demonstrated that eplerenone was well absorbed and rapidly metabolized after oral administration [16]. In our work, the value of $T_{\text{max}}$ was consistent with that found in some previous studies, but the $C_{\text{max}}$ of eplerenone was much higher. It is most likely that eplerenone was dissolved in the sulfobutyl-$\beta$-cyclodextrin solution, which can be a good package for the drug molecule, forming a noncovalent complexes and enhancing the stability and water solubility of eplerenone.

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### Table 3: Stability of eplerenone ($n = 6$).

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Conc. added (µg/mL)</th>
<th>Conc. found (µg/mL)</th>
<th>Accuracy (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard solution</td>
<td>0.20</td>
<td>0.20 ± 0.01</td>
<td>98.33</td>
<td>5.87</td>
</tr>
<tr>
<td>2 h at RT</td>
<td>10.00</td>
<td>9.70 ± 0.53</td>
<td>97.03</td>
<td>5.49</td>
</tr>
<tr>
<td>50.00</td>
<td>47.91 ± 3.82</td>
<td>95.83</td>
<td>7.98</td>
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</tr>
<tr>
<td>Standard solution</td>
<td>0.20</td>
<td>0.19 ± 0.02</td>
<td>96.67</td>
<td>7.90</td>
</tr>
<tr>
<td>30 d at 4°C</td>
<td>10.00</td>
<td>10.05 ± 0.48</td>
<td>100.50</td>
<td>4.78</td>
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<tr>
<td>50.00</td>
<td>47.58 ± 3.25</td>
<td>95.16</td>
<td>6.83</td>
<td></td>
</tr>
<tr>
<td>QC samples</td>
<td>0.20</td>
<td>0.19 ± 0.01</td>
<td>93.83</td>
<td>3.26</td>
</tr>
<tr>
<td>Autosampler</td>
<td>10.00</td>
<td>9.88 ± 0.71</td>
<td>98.83</td>
<td>7.18</td>
</tr>
<tr>
<td>24 h at RT</td>
<td>50.00</td>
<td>48.91 ± 2.07</td>
<td>97.82</td>
<td>4.22</td>
</tr>
<tr>
<td>QC samples</td>
<td>0.20</td>
<td>0.19 ± 0.01</td>
<td>93.67</td>
<td>3.56</td>
</tr>
<tr>
<td>30 d storage at −20°C</td>
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<td>10.18 ± 0.61</td>
<td>101.83</td>
<td>6.04</td>
</tr>
<tr>
<td>50.00</td>
<td>50.24 ± 2.72</td>
<td>100.49</td>
<td>5.41</td>
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<tr>
<td>QC samples</td>
<td>0.20</td>
<td>0.19 ± 0.01</td>
<td>93.50</td>
<td>3.86</td>
</tr>
<tr>
<td>3 freeze–thaw</td>
<td>10.00</td>
<td>10.22 ± 0.59</td>
<td>102.17</td>
<td>5.78</td>
</tr>
<tr>
<td>50.00</td>
<td>48.58 ± 1.74</td>
<td>97.16</td>
<td>3.59</td>
<td></td>
</tr>
</tbody>
</table>

Conc. = concentration; QC = quality control; RSD = relative standard deviation; RT = room temperature.
Figure 3. Typical chromatograms. (A) Reference solution; and (B) Tartarian buckwheat water solution. Peak 1 = rutin; Peak 2 = isoquercitrin; Peak 3 = quercetin; Peak 4 = kaempferol.

Figure 4. Mean concentration–time profiles of eplerenone in rat plasma ($n = 10$) obtained in control group and Buckwheat tea group.
The results of the current study strongly indicate that the dosage of eplerenone should be adjusted in hypertension treatment trials when administered with Tartary buckwheat or Tartary buckwheat-containing dietary supplements to avoid potential drug interactions.

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References