SHORT COMMUNICATION

Comparative Pharmacokinetic Study of Chlorogenic Acid after Oral Administration of Lonicerae Japonicae Flos and Shuang-Huang-Lian in Normal and Febrile Rats

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Shuang-Huang-Lian (SHL), a famous traditional Chinese medicine recipe containing Lonicerae Japonicae Flos (LJF), Forsythiae Fructus, and Scutellariae Radix, is clinically used for the treatment of fever and acute upper respiratory tract infection. In this research, a comparative study was conducted to compare the pharmacokinetic differences of chlorogenic acid (ChA) after oral administration of LJF and SHL to normal and febrile rats with approximately the same dose of 60 mg/kg, and the antipyrexia effect of LJF and SHL on rectal temperature changes induced by Baker's yeast was investigated. The results indicated that $AUC_{0-\infty}$ and plasma concentrations of ChA in the febrile rats were significantly higher than normal rats whether in the extract of LJF or SHL. In addition, SHL increased the values of $AUC$ of ChA in both febrile and normal rats compared with LJF alone ($p < 0.05$), and SHL showed better antipyrexia effect than LJF. These results indicate that fever could play an important role in pharmacokinetic process of ChA. Meanwhile, the combined formula SHL exhibits higher bioavailability of ChA and superior antipyrexia effect than the single herb. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: Lonicerae Japonicae Flos; Shuang-Huang-Lian; chlorogenic acid; fever; pharmacokinetics.

INTRODUCTION

Shuang-Huang-Lian (SHL) is a combined herbal remedy composed of three herbs: Lonicerae Japonicae Flos (LJF) (Lonicera japonica Thunb.), Forsythiae Fructus (FF) (Forsythia suspensa (Thunb.) Vahl), and Scutellariae Radix (SR) (Scutellaria baicalensis Georgi). It is successfully used to treat fever and acute upper respiratory tract infection caused by viruses or bacteria. LJF is highly prized in traditional Chinese medicine practice for its treatment of acute fever, headache, pharyngodynia, respiratory infection, and epidemic disease. Chlorogenic acid (ChA), an ester of caffeic acid with quinic acid, has antibacterial, antiphlogistic, and antioxidant activities (Lou et al., 2009). ChA has been selected as a marker for the quality control of LJF and SHL in Chinese pharmacopoeia. In this paper, ChA was selected as an indicator compound for pharmacokinetic study of SHL and LJF.

It is reported that disease conditions could alter the pharmacokinetics of drugs (Lee et al., 2009). The pharmacokinetic profiles of some drugs were altered during fever (Waxman et al., 2003). SHL is widely used in treating fever and acute upper respiratory tract infection. Several reports have been concerned with the pharmacokinetics of ChA after intravenous administration in health conditions (Gao et al., 2007). A limited number of studies have investigated the pharmacokinetic characteristics of ChA in SHL or LJF when applied in the gastrointestinal tract (Ye et al., 2010; Ren et al., 2007). Although there are a number of pharmacokinetic data about ChA in compound prescription, there seems no information available about the pharmacokinetic profile of ChA in febrile animals or human subjects. Traditional Chinese medicines are administered in disease condition in clinical practice; the pharmacokinetic data in pathological conditions that are valuable for providing the dosing information and are important to the safety and efficacy in clinical applications.

The SHL oral preparations are the most commonly used route in clinical practice. The aim of this study was to investigate the possible pharmacokinetic differences of ChA in febrile and normal rats after oral administration of LJF or SHL and to explore the differences between the single herb and the formula.

MATERIALS AND METHODS

Materials and reagents. The reference standard of chlorogenic acid was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Protocatechualdehyde used as internal standard was obtained from Adamas Reagent Co. Ltd. (Swiss). LJF, FF, and SR were purchased from Nanjing Yifeng drug store, and Yining Lin authenticated these

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decoction pieces. Baker’s yeast (active dry yeast) was obtained from Angel Yeast Co. Ltd. (Hubei, China). HPLC grade acetonitrile was from Tedia Company Inc (Fairfield, OH, USA), and phosphoric acid used as the mobile phase modifier was of analytical grade. Water was purified by a Milli-Q academic water purification system (Milford, MA, USA). All the other reagents were of analytical grade.

**Preparation of SHL and LJF extracts.** The SHL extract was prepared in accordance with the procedure as stated in Chinese Pharmacopoeia (Pharmacopoeia Commission of PR China, 2010). SR, LJF, and FF were mixed in a ratio of 1:1.2. SR was decocted with water three times (1:10, 1:8 and then 1:8, w/v), 2 h for the first time and 1 h for the second and third times. Combined decoctions were filtered and concentrated to a relative density at 80 °C. The pH of the solution was adjusted to 1.0–2.0 with 2.0 mol/L hydrochloric acid at 80°C. Precipitate from the filtered solution mentioned previously was added six to eight times the amount of water, and the pH was adjusted to 7.0 with 40% solution of sodium hydroxide. Ethanol was added until dissolution in the final solution. The pH of the filtrate was adjusted to 2.0 with 2.0 mol/L hydrochloric acid at 60 °C. The precipitate from the filtered solution mentioned previously was further washed with ethanol with the pH adjusted to 7.0. Ethanol was evaporated off under vacuum to obtain SR extract. LJF and FF were macerated in warm water for 30 min, and decocted with water twice (1:10, then 1:8, w/v), 1.5 h for each time. The filtrates from each decoction were combined and concentrated to a thick solution with a relative density of 1.20–1.25 at 70–80 °C. The extract was evaporated in a cold vacuum and combined with the extract of SR, as shown previously. The content of ChA was determined to be 35.45 mg/g with HPLC.

The LJF was extracted with the same procedure as that of SHL, and the content of ChA was found to be 33.49 mg/g.

**Animals and experimental design.** Male and female Sprague–Dawley rats (200–220 g) were obtained from Nanjing Qinglong Experimental Animal Co. Ltd., China. All rats were maintained in an air-conditioned animal quarter at a temperature of 25 ± 2 °C and a relative humidity of 50 ± 10%. They had free access to water and food. Rats were randomly divided into four groups, with six animals in each group, and fasted for 12 h before the experiments. Group I was served as the afebrile normal rat model, whereas group II served as the febrile rat model. The animal experiment in this investigation was conducted in accordance with the internationally accepted principles for laboratory animal use.

The study comprised two experiments as follows:

**Experiment 1.** The normal group was orally administered with LJF or SHL at dosage of 60 mg/kg with ChA. Blood were collected into heparinized tubes before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9, 12 h post-dose. Following centrifugation at 3500 rpm for 5 min, plasma samples were stored at –20 °C until drug assay.

**Experiment 2.** Baker’s yeast was suspended in pyrogen-free 0.9% NaCl. Fever was induced by subcutaneous injection with a pyrogenic dose of Baker’s yeast (2 g/kg).

**Plasma samples analysis.** An aliquot (100 μL) of plasma and 20 μL of internal standard solution (20 μg/mL) were vortex-mixed for 30 s followed by protein precipitation with 400 μL acetonitrile; the mixture was vortexed for 1 min, centrifuged at 3500 rpm for 5 min, then the supernatant was evaporated to dryness, and residual was resolved in 100 μL of mobile phase. A 30-μL aliquot was injected into the HPLC system for analysis.

**Pharmacokinetic analysis.** The Cmax and the time to reach it (Tmax) were obtained from the experimental data. Other pharmacokinetic parameters were evaluated with non-compartmental modeling using a pharmacokinetic software package of DAS (Version 2.1.1, Anhui, China), relating to area under the plasma concentration–time curve, half-life (t1/2), and mean residence time (MRT).

**Statistical analysis.** Statistical analysis was performed using the spss 17.0 software (SPSS Inc, Chicago, IL, USA). All results were expressed as mean ± SD. One-way analysis of variance was used for the statistical comparison. A p-value < 0.05 was considered to be significant.

**RESULTS AND DISCUSSION**

**Effect on Baker’s yeast-induced fever**

The antipyretic effect of SHL and LJF on Baker’s yeast-induced fever is shown in Fig. 1. The administration of Baker’s yeast produced an increase in rat’s body temperature. Eight hour after administration of Baker’s yeast induced fever is shown in Fig. 1.
Chlorogenic acid (ChA), Lonicerae Japonicae Flos (LJF), and Shuang-Huang-Lian (SHL) were evaluated in this study. LJF and SHL extracts, containing chlorogenic acid (ChA), were orally administered to rats at a dose of 60 mg/kg at time 0 h. Values represent mean ± SD (n = 6). *p < 0.01 febrile model compared with normal control, **p < 0.01 LJF group compared with febrile model.

**Pharmacokinetic study**

The plasma concentration–time curve profiles of ChA in normal and febrile rats after oral administration of SHL and LJF are illustrated in Fig. 2. The plasma concentration data were analyzed by non-compartmental methods, and the corresponding pharmacokinetic parameters are presented in Table 1.

Table 1 shows that remarkable pharmacokinetic differences existed between normal and febrile rats when orally administered with SHL, such as $C_{\text{max}}$, $AUC_{0-1}$ and $AUC_{0-\infty}$. In febrile rats, the ChA of plasma showed higher $C_{\text{max}}$ (1.16 ± 0.29 versus 0.42 ± 0.17 μg/mL, $p < 0.01$), $AUC_{0-1}$ (4.45 ± 1.76 versus 2.25 ± 0.72 μg·h/mL, $p < 0.05$), and $AUC_{0-\infty}$ (4.85 ± 1.93 versus 253 ± 0.68 μg·h/mL, $p < 0.05$) as compared with normal rats. However, no significant differences in MRT and $t_{1/2}$ values were observed. Similar to the SHL group, differences were found between healthy and febrile rats in the case of LJF. The $C_{\text{max}}$ and $AUC$ values were higher when fever was induced.

Pharmacokinetic comparison of CHA between LJF and multiherb formula SHL was also conducted in normal and febrile groups. As shown in Fig. 2 and Table 1, the plasma profile demonstrated remarkable increases in the values of $C_{\text{max}}$ (1.16 ± 0.29 versus 0.55 ± 0.21 μg/mL, $p < 0.01$), $AUC_{0-1}$ (4.45 ± 1.76 versus 2.06 ± 0.72 μg·h/mL, $p < 0.05$) and $AUC_{0-\infty}$ after oral administration of SHL in comparison to LJF in febrile rats. Similarly, significant differences in $AUC$ were observed between SHL and LJF in normal rats. These results indicated that some ingredients in FF or SR may affect the pharmacokinetic behavior of ChA.

In the present study, it was found that the pharmacokinetic parameters of CHA had significant differences between normal and febrile rats. Fever could significantly

![Figure 1](image1.png) Antipyretic effect of Lonicerae Japonicae Flos (LJF) and Shuang-Huang-Lian (SHL) on the development of Baker’s yeast-induced fever (2 g/kg). LJF and SHL extract (chlorogenic acid 60 mg/kg) were orally administrated at time 0 h. Values represent mean ± SD (n = 6). *p < 0.01 febrile model compared with normal control, **p < 0.01 LJF group compared with febrile model.

![Figure 2](image2.png) Mean plasma concentration–time curves of chlorogenic acid (ChA) in rats after oral administration of Lonicerae Japonicae Flos (LJF) and Shuang-Huang-Lian (SHL) extract (at a dose of 60 mg/kg ChA) (n = 6). 87 × 59 mm (600 × 600 DPI). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

### Table 1. Pharmacokinetic parameters of ChA in normal and febrile rats after oral administration of LJF and SHL at dose of 60 mg/kg ChA (mean ± SD, n = 6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LJF</th>
<th>SHL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal rats</td>
<td>Febrile rats</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (μg/mL)</td>
<td>0.26 ± 0.08</td>
<td>0.55 ± 0.21*</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.75 ± 0.44</td>
<td>1.12 ± 0.54</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>4.34 ± 1.24</td>
<td>3.07 ± 0.70</td>
</tr>
<tr>
<td>$AUC_{0-1}$ (μg·h/mL)</td>
<td>1.22 ± 0.34</td>
<td>2.06 ± 0.72*</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (μg·h/mL)</td>
<td>1.45 ± 0.35</td>
<td>2.21 ± 0.73*</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>5.95 ± 1.54</td>
<td>4.53 ± 0.55</td>
</tr>
</tbody>
</table>

ChA, chlorogenic acid; LJF, Lonicerae Japonicae Flos; SHL, Shuang-Huang-Lian; MRT, mean residence time.

* $p < 0.05$, ** $p < 0.01$ compared with respective normal rats.

* $p < 0.05$, ** $p < 0.01$ compared with LJF in febrile rats.

* $p < 0.05$ compared with LJF in normal rats.
increase the $C_{\text{max}}$ and $AUC$ values. CHA is metabolized by intestinal microorganisms and liver enzymes to give caffeic acid, m-hydroxyhippuric acid, the glucuronide of m-coumaric acid, and dihydroferulic acid. The metabolites are widely eliminated by the renal route (Stalmach et al., 2010). Fever exerts obvious inhibitory effect on the hepatic drug metabolizing enzymes (Shedlofsky et al., 1994) and produces a marked reduction in glomerular filtration rate (Ismail, 2006). Accordingly, the plasma concentrations and $AUC$ of CHA may be increased as a result of the down-regulated hepatic metabolizing enzymes in febrile rats.

CONCLUSION

In summary, our study showed that pharmacokinetic parameters of CHA had significant differences between normal and febrile rats, regardless of whether in the extract of LJF or SHL. CHA had higher plasma concentrations and $AUC$ value in rats under pathological condition. Higher plasma concentrations may probably be a result of a significantly impaired hepatic microsomal enzyme activity in the febrile rats. Meanwhile, rats orally administrated with SHL had a significant increase in $AUC$ as compared with those rats administered with LJF, adjusted to the same level of CHA. The combined use of the three herbs as SHL formula exhibited superior antipyrexia effect than the single herb.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES


