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The effect of blood stasis syndrome on the pharmacokinetics of hydroxysafflower yellow A in human

Yan-Yan Jia, Jing Yang, Jin-Wen Wang, Yun Tian, Ai-Dong Wen* and Zhi-Fu Yang

Department of Pharmacy, Xijing Hospital, Fourth Military Medical University, Xi'an, China.

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Blood stasis, that is, the decrease of blood flow velocity and the increase of blood viscosity indicates hemorheological abnormalities and pharmacokinetic difference of drugs. Therefore, it is very important to investigate the pharmacokinetics of drugs in patients with blood stasis syndrome, which may influence absorption, distribution, metabolism, and excretion of drugs in blood. The aim of this study was to compare the pharmacokinetics of 140 mg hydroxysafflower yellow A (HSYA) in healthy subjects and patients with blood stasis syndrome due to stable angina pectoris (SAP), and indentify the therapeutic regimen for patients and promote clinical rational drug use. This study was carried out in 12 healthy volunteers and 24 patients with blood-stasis syndrome due to SAP using a single-dose of HSYA (140 mg) under fasting conditions. Venous blood samples were drawn through indwelling cannula from each volunteer prior to drug administration and at 0.5, 1, 1.25, 1.5, 2.0, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, and 15 h after the drug administration. Plasma obtained after centrifuge was analyzed to determine HSYA by high-performance liquid chromatography (HPLC). HSYA pharmacokinetics have a significant difference between healthy subjects and patients with blood-stasis syndrome due to SAP: ratios of C_{max} and AUC_{0-\infty} were 116.5% (105.0 to 134.2) and 132.2% (116.5 to 153.7), respectively; mean terminal half-life (t_{1/2}) was 4.2 h as compared to 2.5 h in healthy subjects. The blood stasis syndrome has an impact on the pharmacokinetic parameters including C_{max}, AUC_{0-\infty}, and t_{1/2}, and dose adjustment should be required for patients with blood-stasis syndrome.

Key words: Hydroxysafflower yellow A, safflower, pharmacokinetics differences, blood stasis.

INTRODUCTION

Safflower yellow injection is a new Chinese drug made of extraction from single herb, Flos Carthamus tinctorius and prepared by Shandong Lvye Natural Medicine Research and Development Center, China, having the effect of activating blood circulation and removing stasis. It is widely used to treat cardio-cerebral-vascular and gynecologic disease. Hydroxysafflor yellow A (HSYA), the main active component of safflor yellow injection, has been demonstrated to have the activities of antioxidation, and myocardial and cerebral protective effect (Chu et al., 2006; Yang et al., 2009). HSYA was chosen as an active marker component for controlling the quality of safflower in Chinese Pharmacopoeia (The State Pharmacopoeia Commission of China, 2005; Nie et al., 2012; European Agency for the Evaluation of Medicinal Products, 2005).

Blood stasis is a pathological state resulting from a sluggish or impeded flow of blood in the body or abnormal blood outside the vessels that remains in the body and fails to disperse (Li et al., 2009). As soon as blood stasis develops, the blood circulation will further be affected and thus lead to new pathological changes. The clinical manifestations of blood stasis vary with the locale and the degree of blood stasis or blood stagnancy, such as abdominal pain, retarded menstruation, dysmenorrheal, dysmenorrheal, thrombosis or pricking pain in the
Pharmacokinetics is the study of what the body does to a drug and human’s physical state has a close relationship to drug transport in human body (Pfeifris, 1999a). As we all know, diseases including hepatic impairment and kidney impairment have significant impact on the pharmacokinetic characteristic of drugs in human (Pfeifris, 1999b, c). Blood stasis, that is, the decrease of blood flow velocity and the increase of blood viscosity indicates hemorheological abnormalities and pharmacokinetic difference of drugs, so it is necessary to investigate the pharmacokinetics of drugs in patients with blood stasis syndrome. Our previous study indicated that the pharmacokinetic characteristic of HSYA has altered in rats with blood stasis syndrome, as compared to the healthy rats. And the pharmacokinetic character of HSYA in healthy Chinese female volunteers has been reported by our team too (Yang et al., 2009). But, there was no report about whether the blood stasis, this especial pathological condition has impact on the absorption, distribution, metabolism, and excretion of drugs in human body.

The aim of this study was to assess the pharmacokinetic characteristic of HYSYA in patients with blood stasis syndrome due to stable angina pectoris (SAP) and healthy volunteers, and to design the rational dose regimen for patients with blood stasis syndrome.

SUBJECTS AND METHODS

This study was conducted in Xijing Hospital, Fourth Military Medical University, China in accordance with the Helsinki Declaration (Kimmelman et al., 2009), and the protocol was approved by the institutional review boards from Xijing Hospital. Written informed consent was obtained from the subjects prior to enrollment.

Study population

This study was conducted in 12 normal subjects and 24 patients with blood stasis syndrome due to SAP. Normal subjects (12) were healthy volunteers. Their health condition was measured based on their medical records and physical examination, vital signs, 24 h electrocardiography (ECG) monitoring, routine clinical laboratory tests (hematology, serum biochemistry, urinalysis, hepatitis B surface antigen screen, hepatitis C antibody screen, and human immunodeficiency virus (HIV) antibody screen). None consumed alcohol or tobacco. No drug was taken, including contraceptives, within 2 weeks during the test.

Patients (24) with blood stasis syndrome due to SAP were enrolled into the study. The diagnosis of blood stasis syndrome due to SAP was based on: conforming to coronary heart disease angina pectoris with blood stagnation syndrome (CHD-AP-BSS) diagnostic criteria, with over 2 episodes of grades I or II; patients differentiated by hemorheological disorders and ultrasonic diagnosis; all the patients enrolled in this study received ECG examination, both general and exercise tolerance test (except those with contraindication), and the load used in the test should be up to elevating or deepening ST segment by 0.05 mV in changes that occurred in general ECG. The result showed: positive figures of general ECG, either during angina episode or not, with ischemic changes (ST segment lower than ≥0.05 mV and/or T wave inverted and deeper than 0.2 mV in R dominative leads); normal figure of general ECG, but positive figures in submaximal exercise test ECG. Patients were excluded if they: had acute myocardial infarction, hypertension (systolic pressure ≥160 mmHg, diastolic pressure ≥100 mmHg), severe cardiovascular dysfunction, or severe arrhythmia (rapid atrial fibrillation, atrial flutter, paroxysmal ventricular tachycardia, etc.); underwent complete vascular reconstruction through coronary bridging or intervention therapy; had serious liver, renal or hematopoietic system diseases, or psychic disease.

All procedures were performed in accordance with the principles of the declaration of Helsinki for biomedical research involving human subjects (Kimmelman et al., 2009). All subjects gave written informed consent before enrollment in the study.

Study design

An open-label clinical study was performed at Xijing Hospital, Fourth Military Medical University. Volunteers were allocated into two groups: the normal control group enrolling healthy volunteers and the experimental group enrolling patients with blood stasis syndrome due to SAP. All subjects were required to fast overnight. A single 140 mg dose of HSYA was given to all subjects by intravenous drip of safflower yellow injection diluted with 150 ml 0.9% normal saline (NS) in 60 min. Blood samples (5 ml) were drawn from antecubital vein and collected into tubes at 0.5, 1, 1.25, 1.5, 2.0, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, and 15 h after administration. All samples were separated and kept at -80°C until analysis. All subjects were prohibited from drinking and participating in vigorous exercise for 24 h before dosing until discharge. No food or drinks was allowed for 4 h after HSYA administration. Smoking and consumption of grapefruit and caffeine-containing beverages were also prohibited during hospitalization. All subjects were discharged on the morning of the day after dosing.

Measurement of drug concentrations

HSYA levels in plasma were measured by high-performance liquid chromatography (HPLC). After the addition of 40 µl of 20 µg/ml riboflavin and 40 µl of 50% methanol to a 200 µl plasma sample, the mixture was stirred immediately for 30 s, and 120 µl of 6% perchloric acid was added, then vortexed for 2 min, and was centrifuged at 12,000 rpm for 10 min. The supernatant (50 µl) was injected into the HPLC for analysis.

The HPLC system consisted of a binary pump (LC-10Atvp, Tokyo, Japan), an ultraviolet (UV) detector (SPD-10A, Shimadzu Corporation, Kyoto, Japan), and a computer system for data acquisition (LC-solution). The systems were operated with LC solution (version 1.21, Shimadzu Corporation), and signals from the UV detector were recorded using the SPD-10A. The detection wavelength was 403 nm. A Shim-pack CLC-ODS C18 column (150 x4.6 mm, inner diameter (i.d.), 5 µm) was used for analysis with a guard column packed with the same packing material. The mobile phase consisted of acetonitrile for pump A, and 0.02 mol/L KH2PO4 adjusted to pH 3.0 with ortho-phosphoric acid for pump B. Gradient elution was employed and the gradient program was used as follows: initial 0 min at 10% solvent A; then 0 to 17 min, linear increase from 10 to 22% solvent A. The system was balanced for 5 min by the initial mobile phase (A:B = 10:90) after detecting each sample. The column temperature was 35°C, and the flow rate was 0.8 ml/min.

Concentrations of standard reference samples for the calibration curve were from 0.04 to 20 µg/ml for HSYA. The within-run and between-run accuracy (relative error) ranged from -1.4 to 2.5% and from 1.2 to 6.8%, respectively, and the within-run and between-run precision (percentage coefficient of variation (CV)) ranged from 1.2...
RESULTS

Demographic characteristics

Twelve healthy subjects (6 males and 6 females) participated in the study (mean [standard deviation, SD] age, 49 [4.2] years [range, 45 to 60 years]; weight, 68.8 [4.5] kg [range, 52.0 to 82.0 kg] and height, 168.6 [6.5] cm [range, 160 to 178 cm]). Twenty-four patients (12 males and 12 females) with blood stasis syndrome due to stable angina pectoris (SAP) also participated (age, 50 [4.9] years [range, 43 to 60 years]; weight, 60.2 [4.4] kg [range, 52.4 to 67.0 kg] and height, 168.9 [6.4] cm [range, 162.8 to 184.9 cm]). There were no significant differences between healthy volunteers and patients in age, height, and sex.

Pharmacokinetic profiles

HSYA pharmacokinetic profiles are as shown in Figure 1. Pharmacokinetic parameter values are summarized in Table 1. The C\text{max} and AUC\text{0\rightarrow\infty} values for HSYA were higher in patients with blood stasis syndrome due to SAP than in healthy subjects. For patients with blood stasis syndrome due to SAP, the elimination t\text{1/2} of HSYA was nearly 4.5 h longer than in healthy subjects. Clearance of HSYA was approximately 25% lower for patients with blood stasis syndrome due to SAP than in healthy subjects. It is suggested that blood stasis syndrome has statistically significant effect on the pharmacokinetic variables of HSYA in patients with blood stasis syndrome due to SAP.

Tolerability

A total of 2 adverse reaction events occurred in 36 subjects; the events were mild in severity and were generally short in duration (resolved within 1 day). Dizziness (1 case) was reported in the healthy volunteers group and a feeling of being hot (1 case) was reported in the patients with blood stasis syndrome due to SAP. No clinically significant changes in physical examination results or vital sign measurements were reported for any subject for the duration of the study. Physical examination results for healthy subjects and patients with blood stasis syndrome due to SAP were normal at all assessments.

DISCUSSION

Blood stasis syndrome, or blood stagnation (Chinese: Xue Yu) is an important underlying pathology for many disease processes according to traditional Chinese medicine (TCM) theory. Described in TCM theory as a slowing or pooling of the blood due to disruption of
Heart Qi, it is often understood in biomedical terms in terms of hematological disorders such as hemorrhage, congestion, thrombosis, and local ischemia (micro clots) and tissue changes (Gunter, 2007). Many researchers suggest that blood stasis syndrome exhibits a close relationship with the development of many diseases, like cardio-cerebral-vascular disease, diabetes II, and even tumor (Xue et al., 2011; Lu et al., 2007; Gouin-Thibault et al., 2001).

Blood stasis, that is, the decrease of blood flow velocity and the increase of blood viscosity indicates hemorheological abnormalities and pharmacokinetic difference of drugs. It is therefore very vital to investigate the pharmacokinetics of drugs in patients with blood stasis syndrome. In this study, the pharmacokinetics of HSYA was different in subjects with blood stasis as compared to healthy subjects. Investigations showed that blood stasis syndrome resulted in the increase of AUC and $t_{1/2}$. The increase in AUC was moderate (>40%) and $t_{1/2}$ of HSYA was about double in subjects with blood stasis syndrome as compared to healthy subjects. It also suggests that the rate and extent of drug metabolism was altered in patients with blood stasis syndrome and the bioavailability was significantly higher in patients with blood stasis syndrome than in healthy volunteers. Therefore, it is recommended that the dose of HSYA should be decreased for patients with blood stasis syndrome, and more attention should be paid on the clinical drug usage in patients with blood stasis syndrome, especially the drugs with narrow therapeutic index or severe toxicity to human body, such as digoxin (Wen et al., 2001). Moreover, therapeutic drug monitoring was needed for some patients with kidney damage, liver injury or blood circulation disorders (blood stagnation).

The number of subjects in this study was a relatively small sample and the digoxin pharmacokinetic difference between patients with blood stasis syndrome and normal subjects has been verified by our study team (Wen et al., 2001). Further research is therefore necessary on the pharmacokinetic character of drugs in patients with various types of blood circulation disease, and the relationship between pharmacokinetic characteristic and blood circulation disease needs to be examined further.

**Conclusions**

This study suggests that the pharmacokinetic profile of HSYA was significantly different between healthy Chinese volunteers and patients with blood stasis syndrome due to SAP. The dosage adjustment is expected to be necessary for patients with serious blood stasis syndrome.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


Table 1. The pharmacokinetic variables of HSYA in 12 healthy volunteers and 24 patients with blood-stasis syndrome due to SAP after administration of 140 mg HSYA injection (mean ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 24)</th>
<th>Healthy volunteers (n = 12)</th>
<th>P-value</th>
<th>Geometric mean ratio</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>16.3 ± 4.4</td>
<td>12.5 ± 4.7</td>
<td>0.04</td>
<td>116.5</td>
<td>105.0 - 134.2</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>1.3 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>0.40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>4.2 ± 0.8</td>
<td>2.5 ± 0.2</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (mg/L/h)</td>
<td>75.2 ± 10.2</td>
<td>48.3 ± 12.8</td>
<td>0.001</td>
<td>131.7</td>
<td>116.6 - 153.2</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt; (mg/L/h)</td>
<td>75.6 ± 14.3</td>
<td>49.5 ± 13.5</td>
<td>0.001</td>
<td>132.2</td>
<td>116.5 - 153.7</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
<td>0.25 ± 0.06</td>
<td>0.35 ± 0.08</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

