Research Paper

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WHETHER CO-ADMINISTRATION OF GARLIC HAS NEGATIVE INFLUENCE ON *SCUTELLARIA BAICALENSIS* GEORGI IN TREATING MODELS RATS WITH PELVIC INFLAMMATION?

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Abstract

The research was to explore whether co-administration of garlic has negative influence on *Scutellaria baicalensis* Georgi *(Scutellaria baicalensis)* in treating models rats with pelvic inflammation. Twelve model rats were randomized into a *Scutellaria baicalensis* group and a *Scutellaria baicalensis*+garlic group with six in each group for pharmacokinetic analysis. Twenty-four rats were randomized into a *Scutellaria baicalensis* group, a *Scutellaria baicalensis*+garlic group, a model control group and a normal control group with six rats in each group for detecting the serum levels of tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) with enzyme-linked immunosorbent assay (ELISA). The results showed that in the *Scutellaria baicalensis* group, the maximum concentration (C_{max}) of baicalin, area under the plasma concentration–time curve (AUC) and the time to C_{max} (T_{max}) were significantly higher and apparent clearance (CL/F) were significantly lower than those of *Scutellaria baicalensis*+garlic group. The serum levels of TNF-α and IL-6 in the *Scutellaria baicalensis* group were both significantly lower than *Scutellaria baicalensis*+garlic group. It was then concluded that garlic not only had negative influence on the absorption of active compounds in *Scutellaria baicalensis*, but decreased the curative effects of *Scutellaria baicalensis* in treating model rats with pelvic inflammation. Garlic should not be co-administered with *Scutellaria baicalensis.*

Key words: *Scutellaria baicalensis* Georgi(*Scutellaria baicalensi*); garlic (*Allium sativum*); pharmacokinetics analysis

Abbreviations: AUC: Area under the plasma concentration–time curve; CL/F: Apparent clearance; C_{max}: Maximum concentration; T_{max}: Time to C_{max}; $t_{1/2(ka)}$: The absorption half time; $t_{1/2(ke)}$: The elimination half time; ELISA: Enzyme-linked immunosorbent assay; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin 6; HPLC: High performance liquid chromatography; AIC: Akaike's information criterion; SC: The Schwarz criterion.

Introduction

Garlic (*Allium sativum*) is a common ingredient in Chinese cuisine. The compounds in garlic have been found to possess beneficial activities for human health (Bocchini et al., 2001). In the clinical practices of Chinese medicine, garlic is often forbidden when patients take anti-inflammatory Chinese medicinal herbs. Studies had shown that garlic decreased the area under the plasma concentration–time curve (AUC) and maximum plasma concentration of saquinavir in volunteers (Piscitelli et al., 2002; Hu et al., 2005). A recent research on investigating the possible impact of two commonly used herbal medicines (garlic and cranberry) on the pharmacokinetics and pharmacodynamics of warfarin found that cranberry significantly increased the area under the international

normalized ratio of prothrombin-time curve by 30% when administered with warfarin and they also found that co-administration of garlic did not significantly alter warfarin pharmacokinetics or pharmacodynamics (Mohammed et al., 2008). However, few researches have been conducted on the influence of garlic on the absorption and the curative effects of anti-inflammatory Chinese medicinal herbs in treating inflammatory diseases. *Scutellaria baicalensi* Georgi has been used for a long time in some Asian countries as a widely-recognized herb with strong anti-inflammatory activities (Kubo et al., 1984). Baicalin, a flavonoid compound purified from *Scutellaria baicalensis*, was often used as an anti-inflammatory agent in the treatment of a variety of inflammatory diseases (Chen et al., 2001). Our study aimed to explore the effects of garlic on the pharmacokinetics and curative effects of *Scutellaria baicalensis* in treating model rats with pelvic inflammation.

Materials and Methods

Scutellaria baicalensis collected from Chengde, Hebei Province, China, was purchased from Huqing Yutang Pharmaceutical Co., Ltd (Hangzhou, China). Fresh garlic was purchased from a local market. Both *Scutellaria baicalensis* and the fresh garlic were authenticated by College of Pharmaceutical Sciences, Zhejiang University. Voucher specimens of *Scutellaria baicalensis* and garlic were deposited in the herbarium of Institute of Material Medica, College of Pharmaceutical Sciences, Zhejiang University, China. Baicalin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The enzyme-linked immunosorbent assay (ELISA) kits of tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) were provided by Boster Biotehnology Ltd., Wuhan, China.

Preparation of the sample solutions

20g of the powdered *Scutellaria baicalensis* was extracted by refluxing with 200ml of distilled water for 60 min, followed by filtration. The same procedure was repeated once. The obtained solution of the extracts of *Scutellaria baicalensis* was combined and condensed to a concentration of 1g/ml. The solution was centrifuged at 3000rpm for 10min.Then the supernatant was filtered through 0.45 μm millipore filter, 20 μL of which was injected for HPLC analysis. The 3D-HPLC chromatogram of the solution of the *Scutellaria baicalensis* extracts is shown in Figure 1. By the HPLC analysis, the content of baicalin in the extracts of *Scutellaria baicalensis* was 104mg/g. 10g of the fresh garlic bulb was dried, powdered and refluxed with 100ml of distilled water for 60 min, also followed by filtration. The same procedure was repeated once. The obtained solution of the extracts of garlic was combined and also condensed to a concentration of 1g/ml.

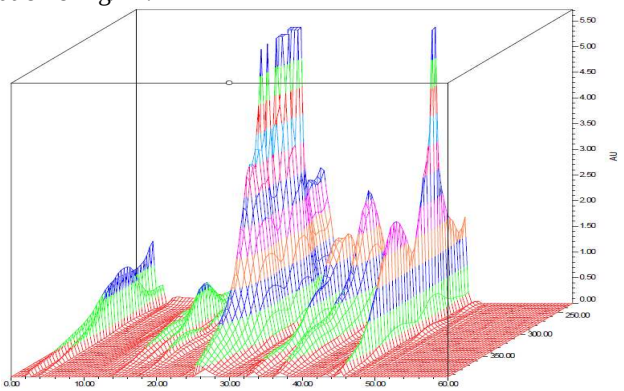

Figure 1: 3D-HPLC chromatogram of the solution of the *Scutellaria baicalensis* extracts

Animals

Thirty-six female Sprague–Dawley (SD) rats (weighing 190±10g) were provided by the Laboratory Animal Center of Zhejiang university (Hangzhou, China). Six were randomly taken out as a normal control group. The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The model rats' pelvic inflammation was established as follows: (1) the abdominal fur was shaved and the skin disinfected; (2) the abdominal cavity was opened; (3) 0.08ml of 20% phenol mucilage was injected into the right uterus of the rats to induce a pathological condition similar to pelvic inflammation (Xiang et al., 2007). (4) the abdominal cavity was then closed. Twelve model rats

were randomly taken for pharmacokinetic analysis, which were then randomized into a *Scutellaria baicalensis* group and a *Scutellaria baicalensis*+garlic group with six in each group. The other eighteen model rats were used for ELISA analysis, which were randomized into a *Scutellaria baicalensis* group, a *Scutellaria baicalensis*+garlic group, and a model control group with six rats in each group. The research was carried out according to the National Research Council's protocol for the care and use of laboratory animals.

High Performance Liquid Chromatography (HPLC) conditions

A diamonsil C18 column (250mm×4.6mm, 5μm) from Dikma Technologies (Beijing, China) was equipped with a pre-column (4mm×5mm) C18. HPLC grade Acetonitrile (MERCK, Germany, as solvent A) and water/Acetic acid (0.8% v/v, pH=6.0; used as solvent B) were used as mobile phase with a linear gradient elution at a flow rate of 0.8 mL/min. The linear gradient elution for *Scutellaria baicalensis* was as follows: 0–10 min, linear gradient 0–5% A. The column temperature was 40°C, and the injection volume of each sample was 20 μL. The solvents were filtered through a 0.45 μm Millipore filter and degassed prior to use.

Preparation of stock and working standard solutions

The standard stock solution of baicalin was prepared by dissolving 30mg of baicalin in 100mL of methanol to obtain a concentration of 0.30mg/mL. The stock solution was kept at 4°C. The standard working solutions of baicalin were respectively prepared at various concentrations of 0.003, 0.03, 0.30, 3.00 and 30.00μg/mL by diluting the stock solution with methanol.

Preparation of the samples

1.0mL of methanol solution was added into 0.2mL of plasma. The mixture was then vortex-mixed for 2 min. After centrifugation at 3,000 rpm for 10 min, the supernatant was filtered through a 0.45 μm membrane filter unit. 20 μL of each sample solution was analyzed by HPLC.

Preparation of calibration standards

The samples used to construct the standard calibration curve of baicalin were prepared by spiking 100μl of the blank rat plasma with 100μL of the appropriate working solutions to yield baicalin concentrations of 0.002, 0.02, 0.15, 1.50 and 15.00μg/mL. The calibration curve was plotted with the concentrations as X-axis and the peak areas as Y-axis using un-weighed linear regression.

Pharmacokinetic analysis

The rats of *Scutellaria baicalensis* group were orally administered *Scutellaria baicalensis* extracts at 0.36g/kg. The rats of *Scutellaria baicalensis*+garlic group were orally administered *Scutellaria baicalensis* extracts at 0.36g/kg and garlic extracts at 0.18g/kg respectively. The blood samples for the analysis of baicalin content were respectively taken from the tail vein at 0.083, 0.167, 0.5, 1, 1.5, 2, 3, 4.5, 7, 9, 12, and 24 h following oral administration of the extracts. The blood samples were immediately transferred into heparinized Eppendorf tubes and were centrifuged at 3000 rpm for 10 min at 4°C, and then the plasma was obtained. All the plasma samples were stored at -20°C until analysis.

The pharmacokinetic parameters were calculated using one or two compartmental methods with 3P87 software (Chinese Society of Mathematical Pharmacology). The AUC was calculated using the trapezoidal method. The half-life ($T_{1/2}$) values were calculated using the equation: $T_{1/2}$=0.693/*K*. The data and the pharmacokinetic parameters were given as mean±S.E. The pharmacokinetic models (one-*vs*.two-compartment) were compared according to Akaike's information criterion (AIC) and the Schwarz criterion (SC). Minimum AIC and SC values were regarded as the best representation of the plasma concentration time course data.

ELISA analysis of TNF-α and IL-6

The *Scutellaria baicalensis* group was orally administered the extracts of *Scutellaria baicalensis* at 0.36g/kg for 7 consecutive days. The *Scutellaria baicalensis*+garlic group was orally administered the *Scutellaria baicalensis* extracts at 0.36g/kg and garlic extracts at 0.18g/kg for 7 consecutive days. The model control group and normal

control group were both orally administered saline at 8ml/kg for 7 consecutive days. When the treatment ended, each of the rats was anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and the serum samples were taken from the caudal vein to detect the levels of TNF-α and IL-6 with ELISA.

Results and Discussion

The regression equations and correlation coefficients (R^2) of baicalin were derived as Y=525509.220X+36344470.09 (R^2=□0.99). The intra-day accuracy ranged from 100.9 to 116.0% (RSD<8%) and the inter-day accuracy ranged from 100.8 to 124.3% (RSD<9%). The Limit of Detection (LOD) of baicalin was between 1 ng to 5 ng. The average recovery of baicalin in *Scutellaria baicalensis*+garlic group and *Scutellaria baicalensis* group was respectively 89.31 % (Relative Standard Deviation (RSD) 6.06%) and 87.44% (RSD 5.89%).

The pharmacokinetic parameters are shown in Table 1. The time courses of baicalin fitted one compartment model. The maximum concentration (C_{max}) of baicalin and time to C_{max} (T_{max}) of *Scutellaria baicalensis* group was respectively 0.13±0.01μg/ml and 2.10±0.37h. The C_{max} and T_{max} of *Scutellaria baicalensis*+garlic group was respectively 0.08±0.01μg/ml and 1.36±0.22h. The values of C_{max} of baicalin in *Scutellaria baicalensis*+ garlic group decreased to 60% of *Scutellaria baicalensis* group. The AUC of baicalin in *Scutellaria baicalensis* group (0.78±0.13 μg·h/ml) was nearly two times higher than that of *Scutellaria baicalensis*+garlic group (0.44±0.09 μg·h/ml).

Table 1: Main pharmacokinetic parameters (mean±S.E.)

parameter	Unit	Values	
		Scutellaria baicalensis group (n=6)	*Scutellaria baicalensis*+garlic group (n=6)
T_{max}	h	2.10±0.37	1.36±0.22
C_{max}	μg/ml	0.13±0.01	0.08±0.01
$t_{1/2(ka)}$	h	1.12±0.14	0.46±0.17
$t_{1/2(ke)}$	h	1.95±0.29	2.47±0.36
AUC	μg·h /ml	0.78±0.13	0.44±0.09
CL/F	ml/h	256.59	451.66

Note: C_{max}: maximum concentration; T_{max}: time to C_{max}; $t_{1/2(ka)}$: the absorption half time; $t_{1/2(ke)}$: the elimination half time; AUC: area under the plasma concentration–time curve; CL/F: apparent clearance.

The plasma concentration–time courses of baicalin of the two groups are shown in Figure 2. There was significant difference between the two groups on the baicalin plasma concentration from 3h to 9h after oral administration with or without garlic. It may be mainly through decreasing C_{max}, T_{max} and AUC, and increasing apparent clearance (CL/F) that garlic had negative influence on the absorption of baicalin in the *Scutellaria baicalensis*.

TNF-α, as one of the major mediators of systemic progression and tissue damage in inflammatory disease, help to propagate the extension of the pelvic inflammatory process (Toth et al. 1992; Frode et al., 2001). IL-6, as an important inflammatory cytokine, has been regarded as a useful adjunct to the diagnosis of pelvic inflammatory disease clinically (Richter et al., 1999). As shown in Figure 3, the levels of TNF-α and IL-6 in all the test drug groups were significantly lower than those of the model control group. The serum levels of TNF-α and IL-6 in the *Scutellaria baicalensis* group were both significantly lower than *Scutellaria baicalensis*+garlic group, suggesting that garlic had negative influence on the curative effects of *Scutellaria baicalensis* in treating the model rats with pelvic inflammation.

All in all, the present research has demonstrated that garlic not only had negative influence on the absorption of active compounds in the *Scutellaria baicalensis*, but decreased the curative effects of *Scutellaria baicalensis* in treating model rats with pelvic inflammation. The present research has provided a positive evidence for the clinical experiences that garlic was forbidden when taking anti-inflammatory Chinese medicinal herbs.

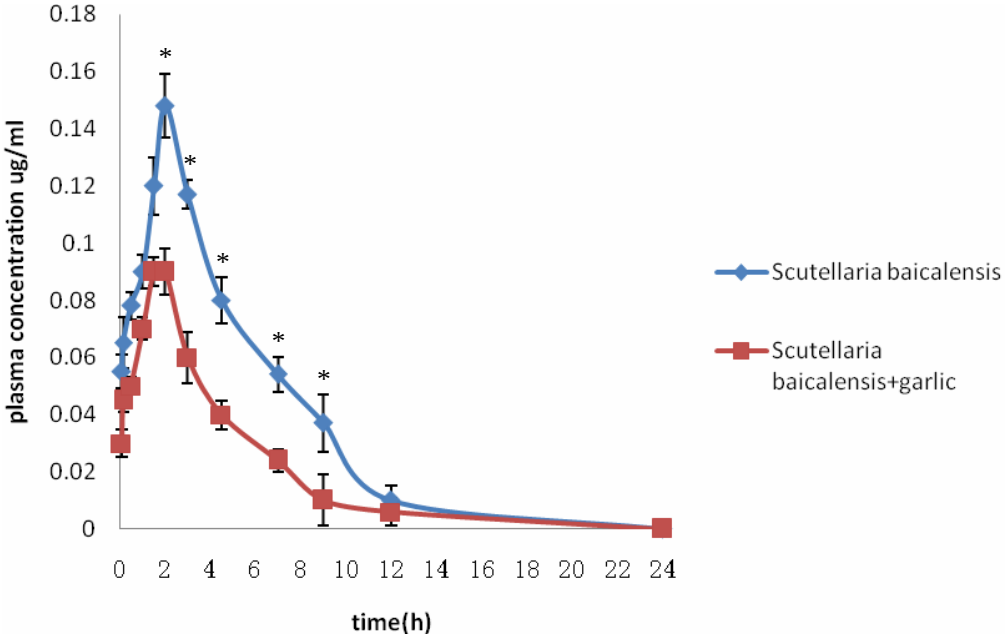

Figure 2: The plasma concentration-time profiles of *Scutellaria baicalensis* group and *Scutellaria baicalensis*+garlic group.
*P<0.05, *Scutellaria baicalensis* group *vs Scutellaria baicalensis*+garlic group.

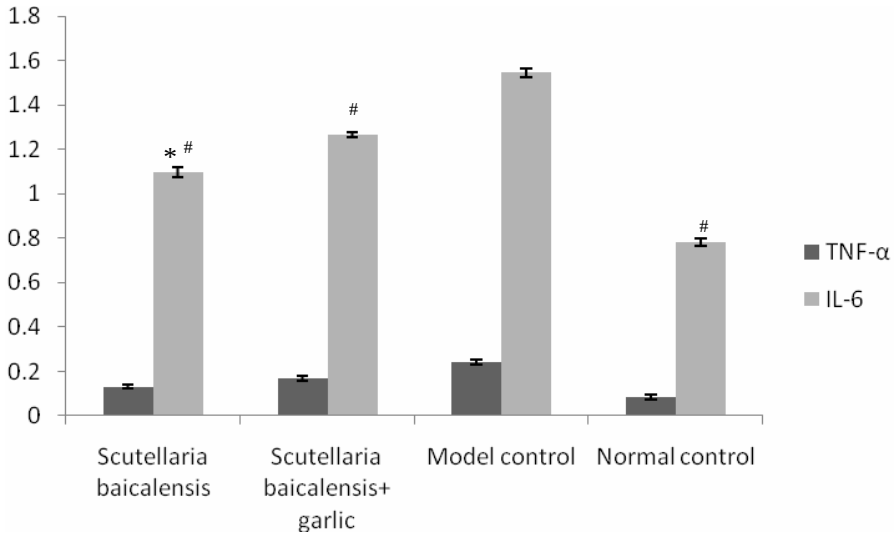

Figure 3: Comparison of TNF-α and IL-6 serum levels
*P<0.05, *Scutellaria baicalensis* group *vs Scutellaria baicalensis*+garlic group;
# P<0.05, *vs* model control group.

Conclusion

Co-administration of garlic not only had negative influence on the absorption of active compounds in

Scutellaria baicalensis, but decreased the curative effects of *Scutellaria baicalensis* in treating model rats with pelvic inflammation.

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