Material Base of *in vivo* Invigorating Qi and Enriching Blood of Bazhen Decoction by HPLC-ESI-MS

LI Wen-lan[1, 2*], BAI Jing[1, 2], SUN Zhi[1], DU Juan[1], JI Yu-bin[1, 2]

1. Center of Research on Life Sciences and Environmental Sciences, Harbin University of Commerce, Harbin 150076, China
2. Engineering Research Center of Natural Anticancer Drugs, Ministry of Education, Harbin University of Commerce, Harbin 150076, China

Abstract: **Objective** To identify the *in vivo* metabolized chemical constituents in Bazhen Decoction (BZD) and to study the relationship between Siwu Decoction (SWD) and Sijunzi Decoction (SJZD) with BZD. **Methods** Analysis and comparison were carried out by HPLC-ESI-MS. Serum samples after ig administration of preparations such as SWD, SJZD, and BZD, with different ingredients were collected for analysis. **Results** Twenty-two components were detected after ig administration of BZD, and six of them were metabolites and others were original form of the components contained in BZD. The prototype constituents were atractylenolide I, 5-hydroxymethyl-furoic acid (5-HMFA), oxypaeoniflorin, atractylenolide III, albiflorin, paeoniflorin, liquiritin, ferulic acid, ligustilide, and ginsenosides Rg_1 and Rb_1. The metabolized constituents were paeonimetabolin I, glycyrrhetic acid mono-glucuronide, glycyrrhetinic acid, and ginsenosides Rh_1 and Rd. **Conclusion** The results also show that 5-HMFA, oxypaeoniflorin, albiflorin, paeoniflorin, ferulic acid, and ligustilide are common components absorbed into blood existing in both BZD and SWD, which are material base of enriching blood; Atractylenolide I, atractylenolide III, and liquiritin are common ingredients absorbed into blood existing in both BZD and SJZD, and they are material base of invigorating Qi. The results provide basic data for the further studies on the effective components, the effecting mechanism, and the quality control of BZD.

Key words: Bazhen Decoction; HPLC-ESI-MS; *in vivo*; metabolites; prototype components
DOI: 10.3969/j.issn.1674-6348.2012.04.004

Introduction

The Bazhen Decoction (BZD) is composed of *Ginseng Radix*, *Atractylodis Rhizoma*, *Paeoniae Alba Radix*, *Angelicae Sinensis Radix*, *Szechwan Lovage Rhizome*, *Rehmanniae Radix*, *Poria*, and *Glycyrrhizae Radix* et *Rhizoma*. It is used for the treatment of weakness of Qi and blood (Peng, Zhan, and Huang, 2000; Zhu and Tan, 2000; Ye and Li, 2008). After ig administration, a part of components are absorbed into the blood and the other parts are directly excreted. On the basis of the fact that the effective components of a drug must be absorbed into the blood, a new method for searching the effective components of drugs directly from serum after taking medicines orally was established.

HPLC-ESI-MS technique has a high sensitivity and selectivity, especially the electrospray ionization (ESI) trap could directly give the excimer ion debris of tested components in a mild condition to verify the relationship between the daughter ions and the parent ions. This provided abundant information not only to identify the chemical components of BZD and those components absorbed into blood, but also to investigate the relationship between Siwu Decoction (SWD) and Sijunzi Decoction (SJZD). It revealed the material base of invigorating Qi and enriching blood. This study has important theoretical significance to illustrate the compatibility principle of herbs.

* Corresponding author: Li WL Address: Research Center on Life Sciences and Environmental Sciences, Post Office Box 48, Harbin University of Commerce, Harbin 150076, China Tel: +86-451-8480 0297 E-mail: lwldzd@163.com
Received: April 18, 2012; Revised: June 13, 2012; Accepted: July 3, 2012
Fund: Postdoctoral Sustentation Fund of Heilongjiang Province of China (LBH-Q07060)

Materials and methods

Experimental equipment

Agilent 1100 HPLC System (including gradient pump, vacuum degassing, automatic sampling, and DAD detector), Agilent 1100 HPLC/VL Type of Ion Trap MS (with ESI source of atmospheric pressure), Equipment Operation, and Data Processing System using Chemical HPLC-3D Workstation, Milli-Q Ultrapure Water for Preparation, TDL80-2B Dove Licensing Centrifuge, XW—80A—Cochlear Rapid Rotation; H66MC-Type Ultrasonic Shock.

Drugs and reagents

Reference substances: paeoniflorin (110736-200320), liquiritin (111610-200402), ferulic acid (0773-9910), ginsenoside Rg_1 (110703-200424), and ginsenoside Rb_1 (110704-200420) were from National Institute for Food and Drug Control. Acetonitrile, methanol (Shandong, China), and formic acid (Shanghai, China) were of chromatographic grade, and homemade ultrapure water and perchlorate (Tianjin Oriental Chemical Factory, China) were used.

Experimental animals

Male Wistar rats (180—220 g, clean grade) were purchased from Laboratory Animal Center of Changchun National Biomedical Industrial Base (China), and Batch No. was SCXK-(Ji) 2003-0004.

Preparation of sample solution *in vitro*

BZD (7.5 g), SWD (4 g), and SJZD (3.5 g) were extracted with methanol twice at 70 ℃, first eight times amount refluxed for 120 min, then six times amount refluxed for 90 min. The combined filtrates were dried under vacuum, dissolved in 10 mL of methanol, then filtered through 0.22 μm membrane filters before HPLC injection.

The reference solution was prepared by dissolving 0.5 mg of paeoniflorin, liquiritin, ferulic acid, ginsenosides Rg_1 and Rb_1 in 2 mL methanol to obtain a nominal concentration of 0.25 mg/mL, then filtered through 0.22 μm membrane filters, and injected into HPLC with volume of 10 μL.

Preparation of solution for ig administration

BZD, SWD, and SJZD were diluted with distilled water to get the final concentration of 3, 1.6, and 1.5 g/mL respectively for ig administration.

Blank serum and serum sample preparation

Wistar rats were fasted for 12 h (water free), divided into four groups, and then ig administered with distilled water, drug suspension of SWD, SJZD, and BZD (20 mL/kg), respectively. One hour after ig administration, all rats were anesthetized with chloral hydrate. Blood (5 mL) was drawn from the hepatic portal vein and stored at room temperature for 30 min, then at 4 ℃ for 2 h. After centrifugation at 3000 r/min for 10 min, the supernatant (2 mL) was precipitated by adding eight times volume of methanol and subsequently centrifuged at 3000 r/min for 10 min. The supernatant was concentrated by rotary evaporation at 37 ℃ to a final volume of approximately 0.4 mL. The solution was filtered through 0.22 μm filtering membrane and was ready for HPLC analysis (Zheng and Song, 2003).

Chromatography and MS conditions

Chromatographic conditions were as follows: column: Waters Symmetry Shield TMRP18 (250 mm × 4.6 mm, 5 μm); $PakC_{18}$ Guard Nova PakTM protect mobile phase: acetonitrile-0.15% formic acid (Table 1); temperature: 30 ℃; flow rate: 0.5 mL/min; detection wavelength: 254 nm; sample volume: 50 μL.

Table 1 Linearity program of mobile system

t / min	Acetonitrile / %	0.15% Formic acid / %
0	2	98
25	15	85
60	21	79
95	33	67
115	40	60
120	80	20

MS conditions were as follows: MS ion source: ESI; atomization air pressure: 0.17 MPa; drying gas flow rate: 10.0 L/min; capillary voltage: 4 kV; transmission voltage: 70 V; scan mode: negative ion scan; scan range: *m/z* 100—1200.

Results

Solution of BZD *in vitro*, blank, and drug-containing serum samples were analyzed under the same chromatographic conditions to identify prototype components and their metabolites (Li, Cheng, and Ji, 2007; Zhang *et al*, 2005; Hu *et al*, 2005; He, Hu, and Cheng, 2006; Yasuda *et al*, 1999).

Twenty-two common components were detected in both BZD and drug-containing serum. Peaks 1—3, 5, 7—10, 12—17, 19, and 22 were prototype components, and peaks 4, 6, 11, 18, 20, and 21 were metabolites.

Because ginseng saponins only had end absorption at 203 nm, we chose 203 nm to detect ginseng saponins. Compared with the t_R and UV spectra of reference substances, peaks 10, 12, 13, 17, and 19 were identified as paeoniflorin, liquiritin, ferulic acid, ginsenosides Rg_1 and Rb_1 (Figs. 1 and 2).

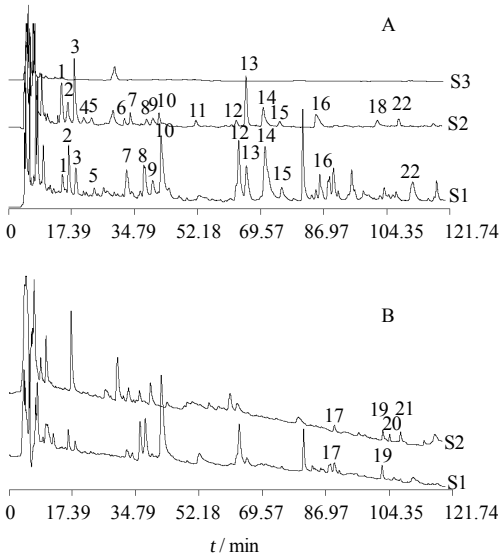

Fig. 1 HPLC chromatograms of BZD (S1), drug-containing serum of BZD (S2), and blank serum (S3) at 254 nm (A) and 203 nm (B)

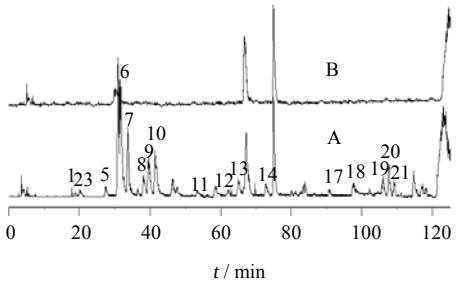

Fig. 2 MS total ion current chromatogram in negative ion mode for drug-containing serum of BZD (A) and blank serum (B)

Based on the recorded *m/z* values, quasi-molecular ion peak, and more fragment information, it is possible to infer the structures of the components.

Secondary ion mass spectrometry analysis on the main debrises was as follows: Peak 1 was atractylenolide I, *m/z* 199 was $[M-H-CH_2O]^-$, *m/z* $[M-CH_2OH]^-$, *m/z* 97 $[M-COOH]^-$; Peak 3 was 214 $[M-H-CH_3]^-$; Peak 2 was 5-hydroxymethylfuroic acid (5-HMFA), *m/z* 111 oxypaeoniflorin, *m/z* 478 $[M-H-OH]^-$, *m/z* 333 $[M-H-Glu]^-$; Peak 5 was atractylenolide III, *m/z* 232 $[M-H-CH_3]^-$, *m/z* 217 $[M-H-CH_2O]^-$; Peak 6 was paeonimetabolin I, *m/z* 151 $[M-H-C_5H_5OH]^-$, *m/z* 108 $[M-H-C_3H_6O_2]^-$; Peak 8 was albiflorin, *m/z* 387 $[M-H-Glu]^-$, *m/z* 431 $[M-H-(\text{benzoic acid})]^-$; Peak 10 was paeoniflorin, 449 $[M-H-CH_3]^-$, 327 $[M-H-CH_2O-(\text{benzoic acid})]^-$; Peak 11 was glycyrrhetic acid monoglucuronide (GAMA), *m/z* 469 $[M-H-Glc]^-$; Peak 12 was liquiritin, 255 $[M-H-Glu]^-$; Peak 13 was ferulic acid, 175 $[M-H-H_2O]^-$, 255 [M−H−Glu]; Peak 14 was ligustilide, 161 $[M-H-H_2O]^-$; Peak 17 was ginsenoside Rg_1, 637 $[M-H-Glu]^-$, 475 $[M-H-2\times Glu]^-$; Peak 18 was glycyrrhetinic acid (GA), *m/z* 438 $[M-COOH]^-$; Peak 19 was ginsenoside Rb_1, 945 $[M-H-Glu]^-$, 783 $[M-H-2\times Glu]^-$; Peak 20 was ginsenoside Rh_1, *m/z* 475 $[M-H-Glu]^-$; Peak 21 was ginsenoside Rd, *m/z* 783 $[M-H-Glu]^-$, *m/z* 621 $[M-H-2\times Glu]^-$ (Table 2).

To sum up, there were 11 prototype components, atractylenolide I, 5-HMFA, oxypaeoniflorin, atractylenolide III, albiflorin, paeoniflorin, liquiritin, ferulic acid, ligustilide, ginsenosides Rg_1 and Rb_1, in serum of rats administered with BZD. There were five metabolites, paeonimetabolin I, GAMA, GA, ginsenosides Rh_1 and Rd, in serum of rats administered with BZD. The metabolic ways were shown in Figs. 3 and 4 (Hang, 2003).

Material base of promoting blood enriching

Solutions of BZD, SWD, and serum samples were analyzed under the same chromatographic conditions (Fig. 5).

Peaks 2, 3, 6, 8, 10, and 13—16 were common ingredients absorded into blood in both BZD and SWD. Peaks 2, 3, 8, 10, and 13—16 were prototype components, and they are 5-HMFA, oxypaeoniflorin, albiflorin, paeoniflorin, ferulic acid, ligustilide, respectively, and peaks 15 and 16 were unknown. Peak 6 (paeonimetabolin I) was a metabolite. All of these components were material base of BZD promoting blood enriching.

Material base of invigorating Qi

Solutions of BZD, SJZD, and serum samples were analyzed under the same chromatographic conditions (Fig. 6).

Peaks 1, 4, 5, 12, 18, and 22 were common components absorded into blood of rats administered with both BZD and SJZD. Peaks 1, 4, 5, 7, 12, and 22

are prototype components, and peaks 1, 5, and 12 were atractylenolide I, atractylenolide III, and liquiritin, respectively, and peaks 4, 7, and 22 were unknown. Peak 18 (GA) was a metabolite. All of these components were material bases of invigorating Qi of BZD.

Table 2 Information of HPLC-ESI-MS and identification of components

Peak No.	t_R / min	*m/z*		Components	Molecular weight
		$[M-H]^-$	MS^2		
1	15.8	229	199 214	atractylenolide I	230
2	17.4	141	11 197	5-HMFA	142
3	18.4	495	478 333	oxypaeoniflorin	496
4	20.8	—	—	—	—
5	23.2	247	217 232	atractylenolide III	248
6	32.3	197	151 108	paeonimetabolin Ⅰ	198
7	33.8	623	441 542	—	624
8	40.4	479	387 431	albiflorin	480
9	41.1	671	323 287	—	672
10	41.8	959	449 327	paeoniflorin	480
11	51.9	545	469	GAMA	546
12	62.5	417	255	liquiritin	418
13	70.6	193	175	ferulic acid	194
14	73.3	191	161	ligustilide	192
15	75.2	827	791	—	828
16	85.0	569	512	—	570
17	88.0	799	637 475	ginsenoside Rg_1	800
18	99.1	483	438	GA	484
19	103.6	1107	945 783	ginsenoside Rb_1	1108
20	104.8	637	475	ginsenoside Rh_1	638
21	107.6	945	783 621	ginsenoside Rd	946
22	108.9	—	—	—	—

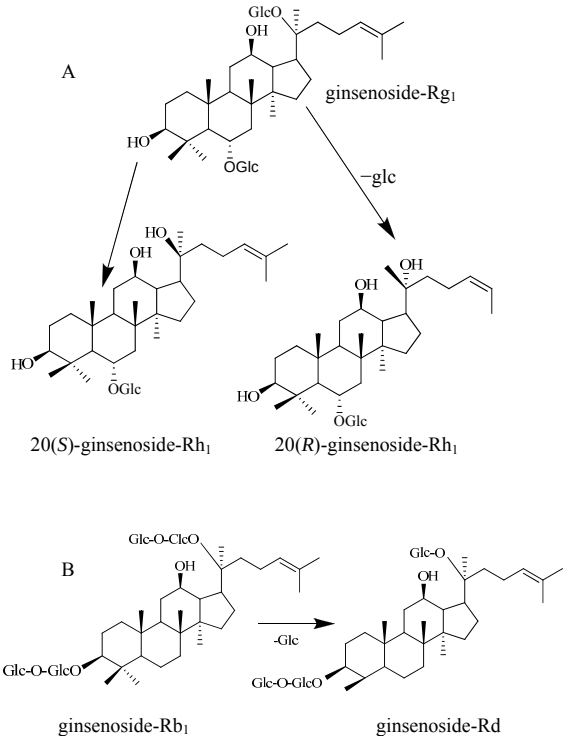

Fig. 3 Metabolic ways of ginsenosides Rg_1 (A) and Rd (B)

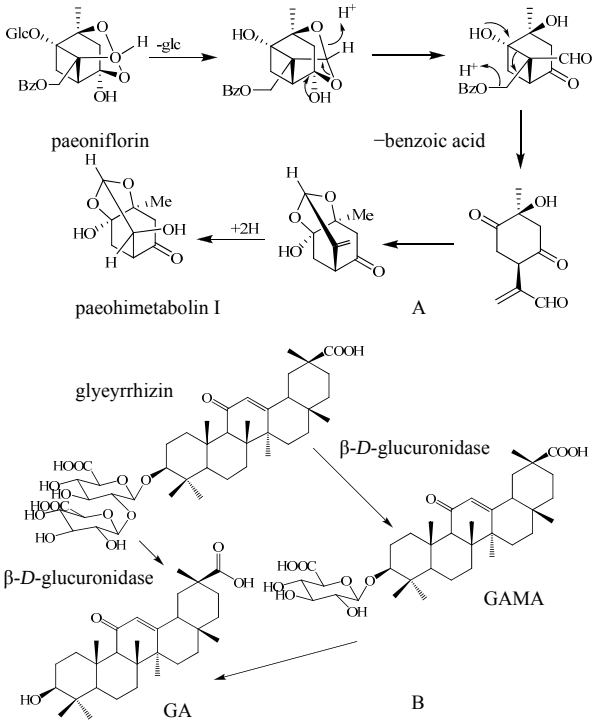

Fig. 4 Metabolic ways of paeonimetabolin I (A) and GAMA (B)

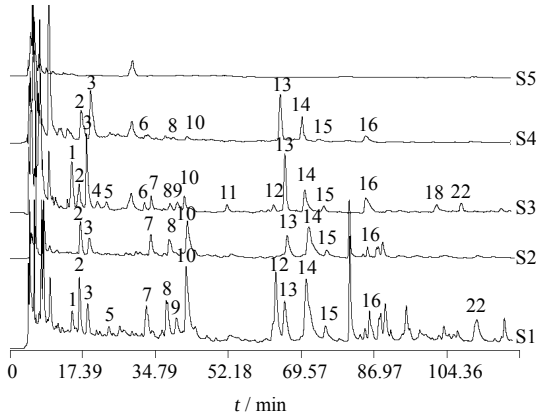

Fig. 5 HPLC chromatograms of BZD (S1), SWD (S2), serum samples of rats administered with BZD (S3), serum samples of rats administered with SWD (S4), and blank serum (S5)

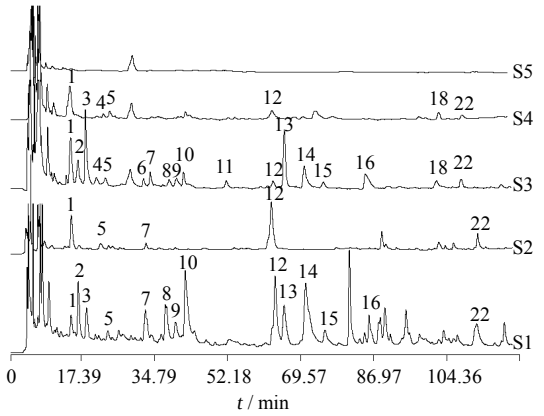

Fig. 6 HPLC chromatograms of BZD (S1), SJZD (S2), serum samples of rats administered with BZD (S3), serum samples of rats administered with SJZD (S4), and blank serum (S5)

Discussion

The study on the active components of Chinese materia medica (CMM) is an essential part for modernization of CMM, and the components absorbed into blood and the metabolites are the possible effective components of CMM. In this study, a method based on HPLC-ESI-MS is proposed to rapidly screen and analyze the multiple absorbed bioactive components and the metabolites of BZD *in vivo*. Twenty-two components are found in the blood of rats after ig administration of BZD. Among them, 16 prototype components are identified as atractylenolide I, 5-HMFA, oxypaeoniflorin, atractylenolide III, albiflorin, paeoniflorin, liquiritin, ferulic acid, ligustilide, ginsenosides Rg_1, Rb_1, and five unknown components; The other six metabolites are paeonimetabolin I, GAMA, GA, ginsenosides Rh_1, Rd, and one unknown component. There are nine chemical components existing in both BZD and SWD and seven among them are identified as 5-HMFA, oxypaeoniflorin, albiflorin, paeoniflorin, ferulic acid, ligustilide, and paeoni- metabolin I. All of these components are basal chemical substances for enriching the blood. There are six chemical components existing in both BZD and SJZD and four of them are identified as atractylenolide I, atractylenolide III, liquiritin, and glycyrrhetinic acid. All of these components are basal chemical substances for invigorating vital energy. This result reveals the chemical composition of BZD and provides the theoretical basis for the further study of pharmacological efficacy of BZD.

References

Hang TK, 2003. *Metabolit Analysis for Chemical*. Chinese Medical Science and Technology Press: Beijing.

He Q, Hu XJ, Cheng YY, 2006. Analysis of "SHUANGDAN" granules by high-performance liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry. *J Pharm Biomed Anal* 41(2): 485-492.

Hu P, Liang QL, Luo GA, Zhao ZZ, Jiang ZH, 2005. Multi-component HPLC fingerprinting of *Radix Salviae Miltiorrhizae* and its LC-MS-MS identification. *Chem Pharm Bull* 53(6): 677-683.

Li WL, Cheng B, Ji YB, 2007. Study on the method of serum sample pretreatment of Jinkuishenqi Pills. *J Heilongjiang Univ*: *Nat Sci Ed* 24(6): 791-794.

Peng HP, Zhan X, Huang DH, 2000. The experimintal study of Bazhen Decoction on red blood cell immunityin mice with blood deficience syndrome. *J Guangxi Med Univ* 17(6): 1015-1017.

Yasuda T, Kon R, Nakazawa T, Ohsawa K, 1999. Metabolism of paeonol in rats. *J Nat Prod* 62(8): 1142-1144.

Ye CX, Li JH, 2008. The influence of Bazhen Decoction in resistance of erythropoietin using to therapy micro-inflammatory state in chronic renal failure with deficiency of both Qi and blood. *Liaoning J TCM* 2(35): 161-162.

Zhang JL, Cui M, He Y, Yu HL, Guo DA, 2005. Chemical fingerprint and metabolic fingerprintanalysis of Danshen injection by HPLC-UV and HPLC-MS methods. *J Pharm Biomed Anal* 36(5): 1029-1035.

Zheng XW, Song H, 2003. Study on effect of Jinkuishenqi Pill on pituicyte generation in kidney-yang deficiency rats. *Chin J TCM Pharm* 7: 413-415.

Zhu HY, Tan YY, 2000. The effect of Bazhen Decoction on the bone marrow cells and related cytokines of mice exposed to ^{60}Co γ-ray. *Chin J Immunol* 2(16): 81-83.