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Contents of four active components in different commercial crude drugs and preparations of Danshen (*Salvia miltiorrhiza*)

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KEY WORDS *Salvia miltiorrhiza*; magnesium tanshinoate B (MTB); high pressure liquid chromatography

ABSTRACT

AIM: To detect the contents of four active components of *Salvia miltiorrhiza* in various commercially available danshen crude drugs and preparations. **METHODS:** Commercially available danshen crude drugs from different sources, as well as danshen pills and intravenous injection preparations containing danshen alone or in combination with other herbs were collected. The composition of these danshen samples was analyzed using HPLC. Specifically, the amounts of magnesium tanshinoate B (MTB), danshensu, isotanshinone II_A, and cryptotanshinone were determined. In some of these samples, the content of MTB was further confirmed by liquid chromatography-tandem mass spectrometer (LC-MS)/MS method. **RESULTS:** There were great variations in the amount of the four active ingredients in the commercially available danshen crude drugs and drug preparations in this study. The amount of MTB was the highest among the four components measured in the crude drugs. However, the amounts of MTB in all danshen preparations were much lower than those in crude drugs. The 2 lipophilic components, isotanshinone II_A and cryptotanshinone, were very low or not detectable in both injection and oral preparations. **CONCLUSION:** MTB can be used to standardize the various forms of danshen crude drugs and drug preparations from different sources. In view of the variation in the amounts of MTB and other components, improvement in the production methods of danshen preparations is essential to ensure consistent amount of its active ingredients and reproducible pharmacological actions.

INTRODUCTION

The Chinese medicine, danshen, is the dried root and rhizome of *Salvia miltiorrhiza* Bge (Labiatae)[1]. Traditionally, danshen is believed to be effective in eliminating blood stasis, relieving pain, promoting blood flow, stimulating menstrual discharge as well as easing the mind[1]. Therefore, it is widely used in many Chinese medicine preparations and formulae. Recent pharmacological studies have indicated that both aqueous and lipid soluble fractions of danshen contain the active components responsible for some of the observed clinical effects. The two active hydrophilic components of danshen are danshensu (Fig 1A) and magnesium tanshinoate B (MTB, also named lithospermic B mag-

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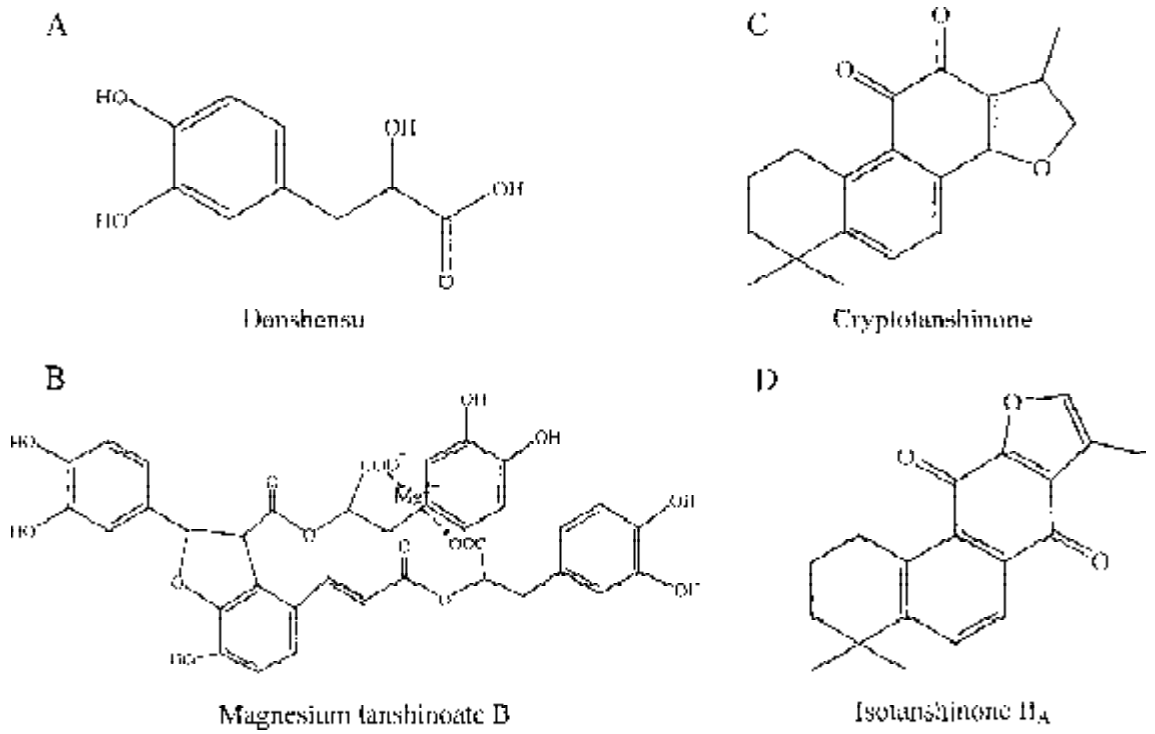

Fig 1. The chemical structures of the four major components of danshen. (A) Danshensu; (B) Magnesium tanshinoate B; (C) Cryptotanshinone; (D) Isotanshinone II_A.

nesium salt, or magnesium lithospermate B) (Fig 1B), while cryptotanshinone (Fig 1C) and isotanshinone II_A (Fig 1D) are the two lipophilic components. These four components are responsible for many of the danshen actions[2]. Among these active constituents, MTB has been found to have strong antioxidative and free radical scavenging effect[3-9]. In addition, MTB has been shown to protect against the pathologic processes of the organ system, such as renal dysfunction, liver damage, and lung fibrosis[10-22]. As a result, there is great interest in the therapeutic potentials of MTB.

Currently, there is great diversity in the source of danshen. Furthermore, preparations may contain danshen alone or in combination with other herbs. This causes confusion regarding the quality of commercially available preparations and the pharmacological effects. In general, the content of isotanshinone II_A has been used as the reference standard for quality control (QC) of crude drugs and preparations. On the other hand, danshensu content has also been used as the reference standard for QC of some of the more recent danshen preparations. In spite of the wide range of pharmacological effects of MTB, the level of MTB has never been reported to be a reference standard for QC. To investigate the composition of danshen crude drugs and preparations in the market, we analyzed the contents of MTB, danshensu, isotanshinone II_A, and cryptotanshinone in various commercially available danshen crude drugs and preparations by HPLC method.

MATERIALS AND METHODS

Equipment The HPLC system consisted of a model LC-10AT pump (Shimadzu, Kyoto, Japan), a model SPD-10Avp UV detector (Shimadzu, Kyoto, Japan) and a model 570 autosampler with a 20 μL loop (Alltech Associates Inc, IL, USA). A chromatography workstation for Windows 95 (Hangzhou Empire Science & Tech Co Ltd, China) was used for data collection.

Reagents Acetonitrile was of HPLC grade whereas methanol, formic acid, and glacial acetic acid were of analytical grade. Deionized and double distilled water was used throughout the study. MTB and sodium danshensu, the internal standards, were provided by Shanghai Institute of Materia Medica, Chinese Academy of Sciences and School of Pharmacy, Fudan University, respectively. Other internal standards, isotanshinone II_A and cryptotanshinone were obtained from the National Institute for the Control of Pharmaceutical and Biological Products.

Sample Sources Crude drugs of danshen were collected from 13 different sources. Nine brands of single and 10 brands of compound Danshen injection preparations were purchased from the Shanghai market. Danshen pills which were used as part of complex prescriptions came from 21 different companies. As well, a total of 14 different batches of danshen tablets from the same company were acquired.

Procedures The chromatographic columns and mobile phases used for analyzing MTB, danshensu, isotanshinone II_A, and cryptotanshinone are shown in Tab 1. All chromatographic separations were performed at room temperature. The flow rate of the mobile phases was 1 mL/min in all cases. Detection wavelengths for MTB and danshensu were 285 nm and 280 nm, respectively. Both isotanshinone II_A and cryptotanshinone were detected at a wavelength of 270 nm.

For the preparations of internal standards and assay samples, water was used as the extraction solvent for water-soluble components (MTB and danshensu), whereas methanol was used as the extraction solvent for lipid-soluble components (isotanshinone II_A and cryptotanshinone). The internal standards MTB, danshensu, isotanshinone II_A, and cryptotanshinone were carefully weighed and dissolved using their respective extraction solvents. They were further diluted to a final concentration of 10 mg/L for MTB, and 4 mg/L for danshensu, isotanshinone II_A, and cryptotanshinone.

Different assay samples were prepared differently according to their physical forms. Danshen crude drugs were dried, grinded, and sifted through 40-mesh screen sieve. Similarly, the fine powder of 20 danshen tablets, 20 capsules, 50 pills, 10 sachets powder and 10 honey bolus forms were carefully weighed out. They were then suspended in an appropriate volume of solvent. Following 20 min of ultra-sonication in a ice bath, the danshen suspensions were filtered. The residues were washed with solvents and removed. The concentration of the resultant solutions were determined and diluted when needed. For liquid form of danshen, appropriate amount was pipetted out from the sources and diluted with solvent to the desired concentrations.

Data are presented as mean±SD and *n* represents the number of preparations or preparations from different brands.

RESULTS

The measurement of the 4 compounds, MTB, danshensu, isotanshinone II_A, and cryptotanshinone, was reproducible by HPLC. The linear correlation coefficients for these 4 compounds were better than or equal to 0.9990 over the range of assay samples used in the present study. Details of the linearity of the individual danshen constituents are shown in Tab 2. Tab 3 shows the precision of the methodology used to deter-

Tab 1. The chromatographic columns and mobile phases used for the analysis of MTB, danshensu, isotanshinone II_A, and cryptotanshinone.

	Chromatographic column	Mobile phase
MTB	Discovery RP-Amide C_{16} (250 mm×4.6 mm, 5 μm)	Methanol-Acetonitrile-Water-Formic acid (42:5:52:1)
Danshensu	Diamonsil	Methanol-Water-Glacial acetic acid (19:80:1.2)
Isotanshinone II_A	C_{18} (250mm×4.6 mm, 5 μm)	Methanol-Water (85:15)
Cryptotanshinone	C_{18} (250mm×4.6 mm, 5 μm)	Methanol-Water (85:15)

Tab 2. Correlation analysis of HPLC detection for MTB, danshensu, isotanshinone II_A, and cryptotanshinone.

	Injected sample amount/μg	Regression and correlation analysis
MTB	0.0761~0.3045	A=-1466+1.844×10^6X, r=0.9996
Danshensu	0.0194~0.1359	A=117.9+145.5X, r=0.9990
Isotanshinone II_A	0.0100~0.6400	A=-146.3+2.830×10^6X, r=0.9998
Cryptotanshinone	0.0100~0.6400	A=5305.8+2.583×10^6X, r=0.9999

Tab 3. The precision and recovery of MTB, danshensu, isotanshinone II_A, and cryptotanshinone in different forms of commercial available danshen samples.

	Precision of assay	Recovery assay					
		Crude drugs		Solid preparations		Aqueous preparations	
	RSD/%	Average/%	RSD/%	Average/%	RSD/%	Average/%	RSD %
MTB	1.61	99.28	1.45	99.99	0.93	102.8	1.41
Danshensu	1.37	98.99	0.97	100.5	0.68	98.08	0.93
Isotanshinone II_A	1.54	99.23	0.72	99.86	0.58	100.3	0.65
Cryptotanshinone	1.67	98.13	0.87	100.1	0.79	98.42	0.97

RSD, relative standard deviation

mine MTB, danshensu, isotanshinone II_A, and cryptotanshinone in different types of danshen samples. The precision of the assay, as represented by the relative standard deviations (RSD), were less than 2 % for all of the samples tested. The average percent recoveries and RSD of MTB, danshensu, isotanshinone II_A, and cryptotanshinone in various danshen crude drugs and preparations were between 98.0 %-102.8 % (Tab 3).

There were great variations in the concentrations of MTB, danshensu, isotanshinone II_A, and cryptotanshinone in different formulations of danshen as illustrated by the large standard deviations (Tab 4). The largest amount of active ingredients detected in

Tab 4. The concentrations of MTB, danshensu, isotanshinone II_A, and cryptotanshinone in different formulations of danshen samples obtained from different sources.

		MTB (mg/MDD)	Danshensu (mg/MDD)	Isotanshinon II_A (mg/MDD)	Cryptotanshinone (mg/MDD)
			(A) Preparation variations		
13 Crude drugs from different sources	Range	52.60–990.3	0.65–23.84	7.2–51.23	1.86–16.11
	Mean± SD	482±254	7±6	20±13	6±4
	RSD/%	52.66	85.79	66.16	66.63
9 Brands of single injection preparations	Range	0.23–28.45	3.16–99.54	ND	ND
	Mean± SD	14 ± 9	57± 31	ND	ND
	RSD/%	64.16	54.08	ND	ND
10 Brands of compound injection preparations	Range	0.22–54.96	6.05–109.9	ND	ND
	Mean± SD	19±17	45±28	ND	ND
	RSD/%	91.75	63.21	ND	ND
21 Brands of compound oral preparations	Range	ND–54.90	0.026–57.82	ND–7.48	ND–5.76
	Mean± SD	10±15	7±13	0.8±2.0	0.4 ±1.3
	RSD/%	152.4	185.6	261.9	350.5
			(B) Batch variations		
14 Batches of one brand of oral preparation	Range	10.15–58.99	9.62–23.76	ND–1.00	ND–0.61
	Mean± SD	25±12	14±4	0.7±0.3	0.41±0.15
	RSD/%	48.41	27.18	45.73	37.69

MDD, mean maximum daily dosage. SD, standard deviation. RSD, relative standard deviation. ND, not detectable

danshen crude drugs was MTB (average amount was 70-, 24-, and 75-fold higher than danshensu, isotanshinone II_A, and cryptotanshinone respectively, Tab 4A). However, MTB amounts were lower than danshensu in the injection forms. Although there were substantial amount of isotanshinone II_A and cryptotanshinone in crude drugs, their levels were very low and even undetectable in some injection and oral preparations. Batch-to-batch variations were also tested in one brand of danshen oral preparation (Tab 4B). Significant variations in both MTB and danshensu were observed while the amounts of lipophilic components, isotanshinone II_A and cryptotanshinone were very low.

DISCUSSION

This study examined the content of 67 danshen samples by means of HPLC. There were 53 danshen samples belonging to 4 different categories: crude drugs, single and compound intravenous injection preparations, as well as pills for oral use containing danshen in combination with other herbs. We had further tested 14 batches of one brand of oral danshen preparation for batch-to-batch variations. The average recoveries of the four major active danshen components, MTB, danshensu, isotanshinone II_A, and cryptotanshinone, were above 98 %, with the precision RSD being less than 2 %. These results, therefore, indicated that our detection methods conformed to the basic requirements for quantitative assessment of crude drugs and drug preparations.

The results from the present study indicated that there were substantial differences in the amounts of the active components among the commercially available danshen crude drugs and preparations. As shown in Tab 4, there were 19- and 37-fold differences in the concentrations of MTB and danshensu, respectively, among the crude drugs of danshen from different sources. Variations in the amount of the four active components as indicated by the RSD values were between 53 % and 86 % for crude drugs and between 54 % and 92 % for intravenous preparations. A RSD of 27 % to 48 % was also recorded for danshen pills that were obtained from the same company.

Differences between crude drugs, and injection and oral preparations may reflect selective extraction and specific processing of danshen in the production of these preparations from crude drugs. In view of the differences in the amount of the 4 major components, it is quite possible that the pharmacological actions of crude drugs be different from that of the injection and oral preparations. For the compound injection preparations of danshen, part of the variation in content may be due to the composition and proportion of danshen and other herbs. Many preparations do not provide clear information on their contents and it is difficult to ascertain whether these preparations have similar therapeutic effects. For the single injection preparation of danshen, the variations in the content cannot be due to the presence of other herbs. Rather, this may be caused by the source of danshen and the manufacturing process. The variations in content for oral preparations of danshen can be due to the reasons mentioned in the above discussion.

One concern in the present study is the large batch-to-batch variation. However, we have only tested different batches from one brand. It is, therefore, unclear whether this is a general finding or this is limited to this particular manufacturer. Nevertheless, it appears that there is a need to improve QC in the manufacture of danshen pharmaceutics.

One limitation of our study is that the actual content of danshen in some of the compound preparations was not clear. As a result, the assays of these samples were expressed as the weights of the active components contained in the maximum daily dosage (MDD) that was specified in the drug usage instruction. For crude drugs and those preparations with known composition, the determinations of the weights of the active compounds could be given in mg per one gram crude drug. However, in order to compare the assay findings of different samples, all the data were calculated with reference to the MDD. The MDD of danshen crude drugs is 15 g as specified of the Pharmacopoeia of the People's Republic of China 2000 Edition.

The average MTB content was 482 mg per MDD in crude drugs. This amount is about 26-34-fold greater than that found in injection preparations, and is 19-49-fold greater than that in oral preparations. Therefore, there are significant reductions in the content of MTB in injection and oral preparations compared to crude drugs. This finding suggests that MTB was lost during the manufacture of various pharmaceutics from crude danshen, and that the current method to process those preparations may require modification if MTB is to be retained.

Recently, there is an intense interest in the

antioxidative and free radical scavenging effects of danshen. Indeed, these actions of danshen have been suggested to be the underlying mechanisms responsible for the pharmaceutical effects. Previous investigations have indicated that MTB is one of the natural components of danshen, and is the major one with antioxidative and free radical scavenging effects. It is expected that the hydrophilic components, such as MTB and danshensu, rather than the lipophilic components, such as isotanshinone II_A and cryptotanshinone, are the predominant ingredients when danshen is processed traditionally by extraction in water. The use of hydrophilic components as reference standards seems more reason-able. Furthermore, we found that the amount of MTB was higher than the other 3 components in crude drugs. Hence, we suggest employing MTB content as the reference standard for QC purpose. This would prevent the production of danshen preparations with low MTB level or highly variable MTB content.

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