Study on the small intestine absorptive kinetics characters of tanshinol and protocatechualdehyde of *Salvia miltiorrhiza* extracts in rats *in vivo*

Kai Liang[1], Shuiting Zhai[1], Zhidong Zhang[1], Guoquan Wang[1], Xiaoyang Fu[1] and Tianxiao Li[2]*

[1]Vascular Surgery, Department of People's Hospital of Zhengzhou University, Zhengzhou, China
[2]Vascular Intervention Department of People's Hospital of Zhengzhou University, Zhengzhou, China

Abstract: In order to provide scientific basis for clinical selection of drugs, to compare and analyze the effective constitutes and the intestinal absorption *in vivo* in rats of the compound salvia tablets and compound salvia dropping pills (taken as the representatives). Determine the contents of tanshinol, protocatechuic aldehyde, salvianolic acid B and tanshinone II A, cryptotanshinone, ginseng saponin Rg1 and Rb1 in the compound salvia tablets and compound salvia dropping pills by High Performance Liquid Chromatography (HPLC). The intestinal absorption condition of the tanshinol, protocatechuic aldehyde, salvianolic acid B of the compound salvia tablets and compound salvia dropping pills in rats were detected by intestinal perfusion experiment. Only the intake of protocatechuic aldehyde in the compound salvia tablets was higher than in the compound dropping pills, the intake of the other 6 effective constitutes were all lower than in the compound dropping pills. The intestinal absorption of protocatechuic aldehyde was rather complete, while the intestinal absorption of tanshinol and salvianolic acid B were not significant. The duodenum was the main absorption region of these three components. The absorption of protocatechuic aldehyde was different in different regions of the intestines. Each intake of the effective constitutes in the tablets and dropping pills were significantly different, and the rat intestinal absorption of part of the components were different.

Keywords: Compound salvia tablets, Reassessment of TCM, Coronary heart disease (CHD), Effective constitutes, Intestinal absorption.

INTRODUCTION

Although Traditional Chinese Medicine (TCM) was developing rapidly, the innovation was not enough in the dosage form changing of generic drugs and there were some serious problems such as low level and repetition. Especially one medicine was in various dosage forms and produced by many pharmaceutical factories with a complex quality. And it lacked comparative study and post-marketing reevaluation, which was harmful to the clinical drug selection and reasonable drug use and need a solution desperately. Fontaine Senate Square complex belonging to the research system side, after many years of clinical validation, its efficacy is significantly and it mainly used in clinical crown heart disease, chest tightness, angina (Mitsuko *et al*., 1983; Su *et al*., 2016; Hu *et al*., 2015).

Compound salvia miltiorrhiza preparation was selected out as the representative to be studied, which was developed and produced by The Secondary Factory of Shanghai Traditional Chinese Medicine in 1977, consisted of Salvia Miltiorrhiza and Pseudo-ginseng and Borneol, with the functions of promoting blood circulation to remove blood stasis and regulazzting Qi to alleviate pain to treat for CHD mainly. At present, there are 10 kinds of dosage forms of compound salvia miltiorrhiza preparation (the tablet, dropping pill, water pill, micro pill, capsule, soft capsule, aerosol, granules, oral liquid, lozenge), produced by more than 700 pharmaceutical factories. The representative tablets and dropping pills were chose out to be comparatively studied and reassessed the pharmaceutical chemistry and pharmacokinetics to provide scientific basis for clinical drug selection.

*Corresponding author: e-mail: zmby519@163.com

MATERIAL AND METHODS

Investigational drugs

Compound salvia tablets (hereinafter referred to as the tablets) were produced by Baiyun Mountain TCM Factory, with three Batch No. G7A026, J7A022 and B8A009. Compound salvia dropping pills (hereinafter referred to as the dropping pills) were produced by Tasly Pharmaceutical Co., LTD. with three Batch No. 20070413, 20070412 and 20071012. The above medicines were all bought from Beijing Haidian Large Pharmacy of Beijing International Biological Products Research, not directly provided by the pharmaceutical factory.

Compound salvia tablets

Prescription of 450g Salvia Miltiorrhiza, 141g Pseudo-ginseng and 8g Borneol, with each tablet weighing 314.2 mg (equivalent to 599mg slice). Clinically each person took 9 tablets per day totally 2827.8mg (equivalent to slices 5.39g/kg). The Clinical equivalent dose of rats was 294 mg/kg, with the dosing volume of 10ml/kg, then the drug concentration was 29.4mg/mL.

Compound salvia dropping pills

Prescription of 90g Salvia Miltiorrhiza, 17.6g notoginseng and 1g Borneol. They were produced into 1000 pills with each weighing 25.8mg (equivalent to 108.6mg slices). Clinically each person took 30 pills per day totally 774.0 mg (equivalent to slices 3.25/kg). The Clinical equivalent dose of rats was 84mg/kg, with the dosing volume of 10 ml/kg, then the drug concentration was 8.4mg/mL.

Experimental animals

SD rats, male, with the weight of 200-220g, were provided by Beijing Charles River Animal Experiment Technology Co., LTD. (Certification No. (BJ) 2007-0001). They were all cleaned and feed by Laboratory Animal House of Xiyuan Hospital of China Academy of Chinese Medical Sciences SPF (experimental animals use License No. SYXK (BJ) 2003-0008).

Reagents

Comparison products: Sodium-tanshinol (110855-200508), protocatechuic aldehyde (110810-200506), Salvianolic acid B (111562-200706), cryptotanshinone (110852-200305), tanshinone II A (110766-200518) and ginseng saponin Rg1 (110703-200726) and ginseng saponin Rb1 (110704-200420), which were all bought from China Pharmaceutical Biological Products Analysis Institute. Methyl alcohol and acetonitrile were all pure chromatography, Honeywell products. Phosphoric acid was pure chromatographic and TEDIA products. The water was ultra pure water. Kreb's-Ringer buffer liquid (Lot No. 10080904), which were all the products of Morning Technology Co., LTD. Sodium carboxymethyl cellulose (Lot No. 930628), Beijing Chemical Reagents Company.

Instrument

High Performance Liquid Chromatograph Instrument with 1100 type series (Agilent Company in the United States), including the quaternary gradient pump of G1311A, automatic sampler of G1313A, column oven of G1316A and detector of G1314AVWD. BT00-300T constant flow pump, was produced by Baoding Lange Constant Flow-pump Co., LTD.

Methods

The comparison of the 7 effective constitutes in the tablets and dropping pills

It was determined by HPLC. Chromatographic conditions as follow: Tanshinol, protocatechuic aldehyde and salvianolic acid B were with the chromatographic column of Agilent SB C18 (5μm, 250mm*4.6mm) and Mobile phase A of 0.1% phosphoric acid aqueous solution and B was acetonitrile. The gradient elution: 0-5 min, 7% B. 5-15 min, 7%-17% B; 15-17 min, 17%-20% B; 17- 21 min, 20%-20% B; 21-37 min, 21% B; 37-45min, 21%-21%B; 45-50min, 29%B. The column temperature was 30°C and the flow rate was 1mL/min. The detection of wavelength was 281nm. The chromatographic conditions of tanshinone II A and cryptotanshinone could be referred to the References (Liu *et al*., 2006). The chromatography of ginsenoside Rg1 and Rb1 was referred to References (Zhang, Shen and So, 2014). The Optimum extraction conditions and the technology was investigated with the method of the References (Zhang, Shen and So, 2014). Which was confirmed feasible, then the practical samples were detected, then the content of the 7 effective constitutes in each intake was counted according to the clinical dose.

Intestinal perfusion experiment in vivo in rats

The tablets and dropping pills were smashed. After that they went through the 100-eyes screen, to be saved in seal to avoid lighting. Perfusion fluid was made up of the buffer solution of the Kreb's-Ringer with 0.5% sodium carboxymethyl cellulose. The tablets and dropping pills were made normal temperature ultrasonic for 30 min before perfusion. Proper perfusion fluid was taken out before and after perfusion. The sample to be tested was prepared by the ultrasonic at room temperature for 30 min with the three times of the volume of 75% methanol aqueous solution (containing 0.1% phosphoric acid). The content of the tanshinol, protocatechuic aldehyde and salvianolic acid B were detected by HPLC, the chromatographic condition was the same as the above. The thermal stability, purfusion pathway adsorption, specificity, linearity relations, precision, repeatability and recovery of the sample were inspected with the methodology before the detection of the practical samples. After confirming of the methodology, the practical samples were detected.

The intestinal tube reflux device *in vivo* in rats were prepared by the method in reference (Sadoogh-Abasian and Evered, 1980). The intestinal content was flushed out at the rate of 5 ml/min, and the intestine was perfused at the rate of 1.0 ml/min. The duodenum segment, jejunum segment, ileum segment and colon segment were selected with the method in the reference (Sadoogh-Abasian and Evered, 1980). The whole intestine perfusion of the duodenum 1cm distant from the pylorus was injected with the perfusion solution and from the colon close to the cecum filling out. Then proper perfusion solution was taken out at present and after 2h, to be prepared for the sample to be tested. After the experiment finished, the length and perimeter of the intestinal segment was detected.

The respectively high, middle and low concentration of the tablets with 53.02mg/mL, 28.60mg/mL and 17.54 mg/mL and the dropping pills with 16.23mg/mL, 9.21 mg/mL, 5.03mg/mL were made with the 1.8 times, 1.0 times and 0.56 times of the clinical equivalent dose. Then the influence of the concentration on the whole intestine absorption of the tanshinol, protocatechuic aldehyde and Salvianolic acid B were detected. The absorption condition in the duodenum, jejunum, ileum and colon

Table 1: The content of the 7 effective constitutes of each dosage of the tablets and dropping pills

Effective constitutes	Intake after taking each medicine (mg/time)	
	Tablets	Dropping pills
Tanshinol	2.17±0. 51	1.89±0.34
Protocatechualdehyde	0.05±0.03	0.65±0.11**
Salvianolic acid B	17.12±1.20	0.45±0.04**
Cryptotanshinone	0.92±0. 08	0.006±0.003**
Tanshinone II A	1.38±0.10	0.008±0.001**
Ginsenoside Rg1	5.45±0.51	1.09±0.07**
Ginsenoside Rb1	5.28±0.67	0.93±0.01**

Compared with the tablets **p<0.01.

Table 2: The heat stability of the perfusion solution in the tablets and dropping pills content of the 7 effective constitutes of each dosage of the tablets and dropping pills

Time	Tablets			Dropping pills		
	Tanshinol	Protocatechual dehyd	Salvianolic acid B	Tanshinol	Protocatechual dehyd	Salvianolic acid B
0min	107.55±1.27	41.74±0.54	1582.05±5.56	93.51±1.42	174.07±3.24	29.58±0.07
30min	105.45±0.50	41.31±0.32	1557.62±14.83	90.08±1.21	179.97±3.00	23.99±0.06
60min	107.66±0.61	42.43±0.33	1592.77±14.332	81.13±2.21	174.71±1.98	16.94±0.65
90min	108.01±0.72	42.51±0.72	1577.24±0.28	72.99±1.53	168.45±2.35	10.98±0.56
120min	104.02±1.03	41.78±0.65	1524.36±9.23	38.51±0.74	149.32±3.01	_
150min	104.01±0.56	42.02±0.79	1522.45±12.32	37.01±0.74	145.12±2.45	_
180min	107.08±0.81	43.41±0.61	1575.61±10.12	34.11±0.82	141.73±2.12	_
RSD (%)	1.5	1.53	1.77	41.29	9.28	_

Table 3: The whole intestinal absorption of rats of the tanshinol, protocatechuic aldehyde and salvianolic acid B in different perfusion solution of tablets and dropping pills (n=6)

		2h total absorptance (%)			The absorption rate per unit area (ng/(cm2*h))		
		High concentration	Middle concentration	Low concentration	High concentration	Middle concentration	Low concentration
Tablets	Tanshinol	6.76±1.15	10.36±0.20	18.53±0.62	1.20±0.26	0.88±0.42	0.70 ±0.12
	Protocatechuic aldehyde	97.12±2.24	100.0±0.02	100.0±0.02	0.85±0.04	_	_
	Salvianolic acid B	5.88±0.77	10.45±1.26	18.57±0.62	9.44±2.49	8.28±0.45	9.13 ±0.93
Dropping pills	Protocatechuic aldehyde	87.03±3.75	92.98±1.20	93.98±1.30	3.27±0.12	1.75±0.16	0.67 ±0.12

were detected with the clinical equivalent dose in rats with drug concentration (tablet 28.60mg/mL, dropping pills 9.21mg/mL). The perfusion fluid density was supposed to be "1" and stayed unchangeable, the volume was expressed with the weight of the perfusion between before-and-after.

STATISTICAL ANALYSIS

All data were expressed in the mean value ± standard deviation. The homogeneity of variance of the data was tested by the Levene's test. If it was equal variance, the comparison among many groups were tested with the One- Way ANOVA (variance analysis of the single factor); if it was significantly different, the comparison was tested by the LSD test. If it was not equal variance, using the Tamhane's T^2 test. $P<0.05$ was statistically significant.

RESULTS

The 7 effective constitutes in the tablets and dropping pills were compared and studied

The dosage was converted based on the drug specification (3 tablets /time, 10 dropping pills/ time). Among the amount of the 7 effective constitutes in the drugs of the patients each time (mg/time), only the protocatechuic aldehyde in the dropping pill was higher than the tablets, the remaining six effective components were lower than the tablets (table 1).

The intake condition of the intestinal absorption in rats of the tanshinol, protocatechuic aldehyde and salvianolic acid B in the tablets and dropping pills

In 37°C water bath for 3 hours, the content of the tanshinol, protocatechuic aldehyde and salvianolic acid B

Table 4: Different intestinal segment absorption of rats of the tanshinol, protocatechuic aldehyde and salvianolic acid B in the tablets and dropping pills (n=6)

The intestinal segments		Tablets			Dropping pills
		Protocatechual dehyd	Salvianolic acid B	Tanshinol	Protocatechualdehyd aldehyde
The intestinal segment with a length 10cm 2h total absorptance (%)	The duodenum	11.08±1.02	77.35±1.70	12.88±1.74	54.30±1.78
	The jejunum	9.22±0.27	69.43±1.83	10.07±3.03	39.39±1.83
	The ileum	5.57±0.42	53.39±1.71	6.03±1.01	45.50±1.07
	The colon	6.59±0.09	46.46±1.86	7.86 ±0.99	34.10±0.53
The absorption rate per unit area (ng/(cm2*h))	The duodenum	5.58±0.92	1.30±0.22	63.34±0.43	6.45±1.20
	The jejunum	4.05±0.75	1.04±0.21	46.90±1.27	3.86±0.55
	The ileum	2.47±0.31	0.84±0.16	27.79±0.25	4.66±0.69
	The colon	3.03±0.49	0.73±0.11	37.68±1.55	3.84±0.28

in the tablets perfusion fluid were not changed obviously. The content of protocatechuic aldehyde in the dropping pills was little changed, while the tanshinol and salvianolic acid B reduced obviously (table 2).

The absorption of protocatechuic aldehyde was rather complete while the tanshinol and salvianolic acid B were not absorbed significantly after the whole intestine perfusion of tablets and dropping pills for 2 hours. The three components were mainly absorbed by the duodenum. The absorption rate of tablets in different intestinal segment: the tanshinol and salvianolic acid B were the duodenum > ileum, jejunum > > colon; While protocatechuic aldehyde: The duodenum > jejunum > ileum > colon. And the absorption of protocatechuic aldehyde was the duodenum > ileum > jejunum > colon (tables 3 and 4).

DISCUSSION

Since there are various components playing pharmaceutical functions in the Chinese herbal medicine compound preparation, it is a key point for the reasonable, stable and standard manufacturing technique to make sure the regent good quality, curative effect and security. As different regent has different manufacturing technique, so do the categories and contents of the chemical components. The raw herbal materials in the tablets and the dropping pills are the same, which are Salvia Miltiorrhiza, Pseudo-ginseng and Borneol with the main treatment for CHD. From the findings of the compared analysis, the contents of the effective constitutes vary differently, whose main reasons are that the ratio of raw herbal materials and manufacturing technique and the clinical dosage are different. According to Pharmacopoeia of People's Republic of China 2005 edition, the tablets were mainly prepared with the reflux and extraction of ethanol, that is, " add ethanol to the Salvia Miltiorrhiza, then heating reflux for 1.5h, then extract filtering medium, then recycle ethanol and concentrate to the defined amount, spare. Add 50% ethanol to the herb residue and heating reflux for 1.5h, extract filtering medium, then recycle ethanol and concentrate to the defined amount, spare. Add some water to the herb residue and boil for 2h, flirt the decoction solution, the extraction concentrate to the defined amount. Smash Pseudo-ginseng into fine powders, mix with the above concentrated solution and appropriate auxiliary materials into grains, then dry. Porphyrize the Borneol, mix up with the grains (China Pharmacopoeia Committee, 2005), then compressed to the tablets.

Therefore, tablets consist of liposoluble constituents and water-soluble ingredients of the Salvia Miltiorrhiza and the whole components of the Pseudo-ginseng. While the manufacturing technique of the dropping pills is mainly the water boiling and precipitation with ethanol, that is "add some water to the Salvia Miltiorrhiza and Pseudo-ginseng. Then boil out. Filter the decoction solution, extract the filter liquor, then add ethanol, stand and precipitate. Take the supernatant, recycle ethanol, then condensed into the thick paste form, spare. Porphyrize borneol. Take proper polyethylene glycol, heat into fusion, add the above thick pastes and porphyrized borneol and mix them up, then drop them into the cold liquid paraffin, or film-coating and the dropping pills are made out" (China Pharmacopoeia Committee, 2005). The dropping pills mainly extract the water-soluble ingredients in the Salvia Miltiorrhiza and Pseudo-ginseng, while the various biological activities of the liposoluble constituents of the Salvia Miltiorrhiza (such as cryptotanshinone and tanshinone II A) are not made the best use (Liang, Yang and Yuan, 2000; Wei and Zhang, 2004; Lee *et al*., 2014). Lee *et al* (2014) compared the contents of the tanshinone II A, salvianolic acid B, protocatechuic aldehyde, panax notoginseng saponins R1, ginseng saponin Rg1 and ginseng saponin Rb1 and the cryptotanshinone in the tablets in three factories and the dropping pills with 3 Lot. No., they found that only the protocatechualdehyde in the dropping pills were higher than the tablets, while the other 6 components were lower than the tablets. In the research of Pang *et al* (2016), it was found that the content of the salvianolic acid B and tanshinone II A were 9.25-9.80mg/tablet and 0.29-0.32 mg/tablet respectively. While

they were not detected in the dropping pills. Huang *et al* (2006) also found that the content of the total phenolic acids with water-soluble in the tablets were obviously higher than the dropping pills. The findings in this study were that only the protocatechualdehyde in the dropping pills were higher than the tablets, while the other 6 components were lower than the tablets. Which was the same as the above (Lee *et al*., 2014; Pang *et al*., 2016; Cao *et al*., 2009).

According to the specification of clinical dosage, the dosage of tablets (calculated on slices) were nearly 1.6 times higher than the dropping pills. Therefore, the intake amount of the 6 effective contents were all lower than the tablets, in which the intake of the cryptotanshinone and tanshinone II A in tablets are thousands higher than the dropping pills. A large number of studies had shown that tanshinol, protocatechuic aldehyde, salvianolic acid B, tanshinone II A, cryptotanshinone, ginsenoside Rg1 and ginsenosides Rb1 could inhibit the aggregation of platelets, promote the formation of the antithrombus and oxidation resistance and protection of vascular endothelial cells, and other functions (Song *et al*., 2015; Li, Qian and Zhao, 2016). The difference of effective constitutes in each medication intake led to the different pharmacological effects and therapeutic effects in the two drug products.

Rats *in vivo* intestinal perfusion technology is the simple, fast and feasible model to study traditional Chinese medicine (TCM), widely used in these fields such as the absorption of TCM, *in vivo* pharmacokinetic characteristics and herbal formulation (Zhai *et al*., 2014). This study made a research on the intestinal absorption of the tanshinol, protocatechuic aldehyde, salvianolic acid B used the rat *in vivo* intestinal perfusion technique. It was found that the intestinal absorption of protocatechuic aldehyde in different intestinal segments were different. And the co-ingredients and the auxiliary materials in the drugs might affect the results.

Tablets and dropping pills zhongyuan catechu aldehyde in different absorption of the intestine has certain differences, medicine the coexistence of other ingredients and may affect the absorption of accessories.

CONCLUSION

Compound preparations of TCM are a complex of a variety of composition, with the inter-influence of each effective components in the body, co-playing the curative effect. With the change of the production technology, the remaining ingredients in the preparations have changed, which further influence the pharmacological effects of drugs and metabolic processes in the body. Therefore, the research and development of new drugs of TCM, especially the research and development of changing dosage form should be fully considered the influence of the manufacturing technique and dosage forms on the effective constitutes of TCM. There for, a reasonable amount of dosage should be chose to avoid weakening the pharmaceutical effects.

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