Int J Clin Exp Med 2017;10(10):14614-14622
www.ijcem.com /ISSN:1940-5901/IJCEM0055725

Original Article

Clinical research of yang xin tang's treatment on obstruction of the heart qi deficiency syndrome by impacting metabolic pathway of 5-LOX

Xiaohong Yu*, Yan Wang*, Jing Sun, Yabin Zhou

*Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, China. *Equal contributors and co-first authors.*

Received April 18, 2017; Accepted June 22, 2017; Epub October 15, 2017; Published October 30, 2017

Abstract: Objective: To investigate the possible therapeutic effects of yang xin tang on unstable angina (UA), study the related onset molecule that may cause UA and further analyze the mechanism of yang xin tang's treatment on UA by the metabolomics of High performance liquid chromatography mass spectrometry (HPLC-MS/MS). Methods: Random number table method was applied. One hundred and twenty UA patients were divided into group with traditional Chinese medicine treatment (M group, n=60) and group without traditional Chinese medicine treatment (N group, n=60). In the end of the study, only 103 patients (53 in M group, 50 in N group) completed the clinical research because of some irresistible factors. As for control group (C group), 50 healthy volunteers were selected. The traditional Chinese medicine (TCM) symptoms, angina pectoris symptoms, improvement of electrocardiogram (ECG), and the stopping or reducing condition of nitroglycerin in M group and N group after the treatment were observed and recorded. The HPLC-MS/MS was applied to analyze possible UA-related onset molecule and the RT-PCR was used to detect the expression of 5-LOX mRNA. Results: Compared with N group, improvement rate of angina pectoris symptom (P=0.019), that of TCM symptoms (P=0.042) and that of ECG (P=0.037), reduce rate of nitroglycerin stop (P=0.040) in M group significantly increased. The differences were statistically significant. Analyzed by HPLC-MS/MS, LTB4 was found to be the potential marker of UA. Meanwhile, ELISA results showed the LTB4 contents of plasma in M group and C group were lower than those in N group (both P<0.001). Results of RT-PCR indicated that the mRNA expression of LTB4 catalytic enzyme and 5-LOX of plasma in M group and C group were lower than those in N group (P=0.004, P=0.003 respectively). The differences had statistical significance. Conclusion: Adverse reactions caused by UA can be significantly improved by yang xin tang. Its possible mechanism is to treat UA by exerting an influence on the metabolic pathway of 5-LOX and then reducing the production of LTB4, so as to have therapeutic effects on UA.

Keywords: Yang xin tang, obstruction of the heart qi deficiency syndrome, 5-LOX, metabolic pathway, treatment strategy

Introduction

Obstruction of the heart qi deficiency syndrome in traditional Chinese Medicine (TCM), which is also named unstable angina (UA), is a kind of clinical syndrome between stable angina pectoris and acute myocardial infarction (AMI), clinical instability of which mainly depends on the instability of local lesion in coronary artery [1]. Specifically, it refers to the degrees, frequencies and inducing factors of pain of the patients with original stable angina pectoris change constantly in three months, exacerbate progressively or recover to be stable [2]. It can develop into AMI rapidly and even lead to sudden death. Today UA has become one of the major diseases leading to death and posed a serious threat to human health [3].

Chinese herbal compound yang xin tang is the result of Professor Zhou Yabin according to his years of abundant clinical experience and extensive expertise. It is processed on the basis of ancient prescriptions yang xin tang, which consists of astragalus membranaceus, poria with hostwood, Chinese angelica, ligusticum wallichii, ginseng, spina date seed, schisandra chinensis, fresh ginger, platycladi

seed, red dates, dried tangerine peel, pinellia tuber, polygala tenuifolia, radix glycyrrhizae and spicy cinnamon. Modern research has confirmed its treatment effects on the improvement of myocardium. Yang xin tang can increase the content of 6-Keto-PGF1αα in plasma and reduce the production of TXB2 by adjusting the function of vascular endothelium [4]. Thereby, it can protect ischemic myocardium and prevent the increase of the thickness of artery caused by high fat diet [5]. Besides, it can significantly prevent LDL-C, TG and TC from increasing and obviously increase HDL-C to regulate the abnormity of blood fat [6]. Yang xin tang can increase the production of NO and NOS to protect ischemic myocardium by adjusting the function of vascular endothelium [7].

This research was designed to investigate the therapeutic effects of Chinese herbal compound yang xin tang on UA, study the related onset molecule that may cause UA, thereby further analyzing the mechanism of yang xin tang's treatment on UA through the metabolomics of HPLC-MS/MS.

Materials and methods

Diagnostic standard and TCM syndrome differentiation standard

Western medicine diagnosis standard referred to *Nomenclature and standard for diagnosis of ischemic heart disease*, which meant all the types of angina were UA except typical stable exertional angina. The features were as follows: 1) the angina was stable angina pectoris originally, and its frequency of pain attack within one month increased, its degree of pain attack aggravated, its time limit extended, its inducing factors changed, and its alleviation of nitrate drugs weakened; 2) the new angina occurred within one month and it was induced by light load and occurred at rest or induced by light activities; 3) electrocardiogram (ECG) showed that the downward segment of S-T segment was more than or equal to 0.05 mV, or T wave inversion was more than or equal to 0.1 mV. Variant angina pectoris occurring with the raise of S-T segment also can be included [8]. TCM syndrome differentiation standard referred to TCM syndrome diagnostic standard related to coronary heart disease and angina pectoris included in *Guiding principles of clinical research on new drugs of traditional Chinese medicine* [9]. And it also referred to *TCM syndrome differentiation standard of coronary heart disease* revised by cardiovascular society of integrated traditional Chinese and western medicine [10]. Pathogenesis of obstruction of the heart qi deficiency syndrome included little blood deficiency, qi deficiency, blood running weakness, venae stasis and mind dystrophy.

Inclusion and exclusion criteria

This study was approved by the medical ethics committee of our hospital and patients and their family members has signed informed consent.

Inclusion criteria: Patients who were corresponded to diagnosis standards, aged 40-70; the UA conformed to diagnostic standard of western medicine and TCM diagnostic criteria; the resting ECG was positive [the S-T segment depression ≥0.05 mV and (or) R wave as main lead, T wave inversion and its depth >0.1 mV; patients who signed informed consent willingly.

Exclusion criteria: The patients with chest pain caused by diseases like stable exertional angina, AMI of coronary heart disease or other heart diseases, climacteric syndrome, neurosis, hyperthyroidism, hiatus hernia, cervical spondylosis, aortic dissection or some other disease; patients with uncontrolled hypertension (systolic pressure ≥160 mmHg or diastolic pressure ≥100 mmHg), severe cardiopulmonary insufficiency, complete bundle branch block, diabetes severe complications or severe arrhythmia; patients with combined serious damage of kidney, liver, hemopoietic system and other body parts; the gestating or lactating women; patients with disability (deaf, blind, dumb, mental disorder, intelligence disorder or extremity disability); patients who were suspected or confirmed to have a history of drug or alcohol abuse; patients with allergic constitution (who were allergic to two or more kinds of drug or food); patients who joined other clinical research of medicine within the past one month.

General information

One hundred and twenty patients were all UA patients in TCM department of our hospital

Table 1. A comparison of general information of M group, N group and C group

Group	Sex		Age (years old)	Course of disease			NYHA heart function classification			Education			
	Male	Female		<5 years	5-10 years	>10 years	II	III	IV	No	Primary school	Middle school	College
M group (n=53)	27	26	60.72±1.21	15	18	20	14	23	16	10	21	13	9
N group (n=50)	28	22	59.68±1.33	18	17	15	16	20	14	11	15	18	6
C group (n=50)	24	26	59.91±2.01	-	-	-	-	-	-	9	15	16	10
χ^2/F	0.66		0.59	0.93			0.39			3.68			
P	0.720		0.553	0.629			0.823			0.720			

from December 2013 to December 2016. The heart function was classified from II to IV grade. All patients were divided into traditional Chinese medicine treatment (M group, 60 cases) and group without traditional Chinese medicine treatment (N group, 60 cases) using random number method. Due to some irresistible factors, only 103 cases of patients completed the clinical research in the end. And there were 53 cases in M group (male 27, female 26, aged 60.72±1.21) and 50 cases in N group (male 28, female 22, aged 59.68± 1.33). Besides, 50 healthy volunteers were collected in control group (C group) (male 24, female 26, aged 59.91±2.01). The general information, such as course of disease, heart function classification and degree of education was compared (see **Table 1**). The differences had no statistical significance.

The prescription of TCM

This prescription was composed of 15 kinds of TCM like ginseng and astragalus membranaceus on the basis of traditional prescription of ancient yang xin tang. The ancient yang xin tang originated from *Standard of diagnosis and treatment· categorized formula·discomposure* written by Wang Kentang of Ming Dynasty. The yang xin tang was mainly for the treatment of exhausted heart with slow blood slow and atrial fibrillation, its prescription was composed of poria with hostwood (wood removed), white poria (peel removed), astragalus membranaceus (roasted), pinellia tuber fermented medicinal, Chinese angelica, spina date seed (peel removed, stir-fry on the paper laid on the pan until perfuming), ligusticum wallichii (1.5 Qian each), polygala tenuifolia (kernel removed, soaked in ginger juice and baked), platycladi seed, spicy cinnamon, schisandra chinensis, radix glycyrrhizae (roasted, 0.5 Qian), ginseng (1 Qian), two cups of water, two red dates and five slices of ginger (Qian is a measurement unit in ancient China, 1 Qian is about 3.125 g). All these ingredients were boiled to one cup of liquid which should be taken before meals. The red poria and betel nuts were added for the treatment of slow internal water circulation and severe palpitation. In this prescription, ginseng and astragalus membranaceus were applied as primary ingredients (so-called monarch drug). These two kinds of medicine ingredients are both beneficial to lung and spleen and they also have tonic effects. Besides, red poria and betel nut are applied as supplements. These two kinds of medicine ingredients both are beneficial to heart and spleen and they also have tonic effects on the central body. Chinese angelica and ligusticum wallichii are also employed as supplements in activating blood, nourishing blood and promoting Qi.

Treatment methods

Only regular western medicine treatment was used in N group. While in M group, both regular western medicine treatment and yang xin tang (150 mL/bag, prepared by the pharmacy of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine) were applied. Yang xin tang was taken once for every morning and evening for 28 continuous days. As for C group, no treatment was applied.

Observation of curative effects

After treatment, patients' improvements of TCM symptoms, angina pectoris, nitroglycerin reducing or stopping and ECG should be observed immediately. TCM syndrome curative effect judgment standard: curative effect index (n) = (the total score before treatment - the total score after treatment)/the total score before

treatment *100%. When clinical symptoms and signs were obviously improved, and the curative effect index (reduced score value) ≥30%, it meant effective. When the clinical symptoms and signs were not improved or became even worse or curative effect index (reduced score value) <30%, it meant ineffective. The angina pectoris symptoms improvement standard: when the symptoms disappeared or mostly disappeared or the degrees, times and lasting time of effective pain obviously reduced, it meant effective for angina pectoris I. When the symptoms disappeared or mostly disappeared or reduced to standard of degree I, it meant effective for angina pectoris II. When the symptoms disappeared or mostly disappeared or reduced to degree II, it meant effective for angina pectoris III. When the symptoms after treatments were as same as ones before treatments or became even worse, it meant ineffective. The standard of nitroglycerin reducing or stopping: when the nitroglycerin was completely stopped or reduced after treatments compared to the dosage before treatment, it meant reduced or stopped. When the reduced dosage of nitroglycerin was less than 50% or the dosage was even more compared to the dosage before treatment, it meant same or increased.

The curative effect standard of ECG: as for effective cases, the patient's ECG was recovered to "roughly normal" or reached to "normal ECG". Or the declined S-T segment was rebound for more than 0.05 mV but did not reach the normal level, or the R wave was the main lead and the inverted T wave became shallow (more than 25%). Or flat T wave got upright or the symptom of atrioventricular or intraventricular conduction block was improved. As for ineffective cases, the patient's ECG was basically same or S-T segment was declined for more than 0.05 mV when compared to ECG before treatments. Inverted T wave was deeper (for more than 25%) or the upright T wave got flat or flat T wave became inverted. Or the symptoms of ectopic cardiac rhythm, atrioventricular block and intraventricular block appeared.

The collection and conservation of plasma sample

First, draw 2 mL elbow vein blood from UA patients who finished fasting for great than or equal to 10 h. And use LEDTA for anticoagulation. Then put the collected plasma into eppendorf tube of 1.5 mL after centrifuged at 3000 r/min for 15 min. Keep the Eppendorf tube at -20°C for one week and then transfer it to refrigerator which is -80°C. Draw blood from patients of treatment group before and after treatment while draw once from the healthy volunteers before entering group.

Pre-test sample processing

The preserved plasma was naturally thawed and then mixed by a liquid fast mixer for 30 s and transferred to a high speed centrifuge at 10000 r/min centrifuged for 2 min. And 20 μL plasma was removed from the original sample, placed in a newly-marked EP tube, and then 10 times with 180 μL methanol dilution being added to the new tube. It was mixed by using a liquid fast mixer for 30 s, and then the mixed liquid was placed in a centrifuge and centrifuged at 13200 r/min for 5 min, and then 100-150 μL plasma was put into a test bottle for testing.

HPLC-MS/MS full scan test condition

Instruments [11]: API (spray ion source), LC-MS/MS 3200; mobile phase B, 10 mmol/L ammonium acetate; 0.01% formic acid; mobile phase C: acetonitrile chromatographic column: Eclip XDB C18 46 mm *150 mm, 5 μm, Aglient; sample volume: 5 μL; mass spectrometric parameters: CUR: 25.00; GS1: 50.00; GS2: 60.00; TEM: 550.00; DP: -50.00; EP: -10.00; ihe: ON; IS: -4500.00.

Enzyme linked immunosorbent assay (ELISA)

Detection of LTB4 content in plasma: Venous blood samples of patients in M group, N group, C group were collected and placed in room temperature for 2 hours, and then 100-μL blood serum was collected. The expression levels of LTB4 in blood serum were determined by LTB4 ELISA Kit (R&D Company, USA).

Real time fluorescent quantitative RT-PCR

The expression level of 5-LOX mRNA was detected as follows: 1) total RNA of M group, N group, C group were extracted: 4 mL fresh venous blood was extracted from patients in M group, N group and C group respectively, and

Table 2. UA treatment comparison between M group and N group (n, %)

Group	Angina pectoris	TCM	ECG	Nitroglycerin stopping and decrease
M group (n=53)	51 (96.23%)	52 (98.11%)	40 (75.47%)	35 (66.04%)
N group (n=50)	41 (82.00%)	44 (88.00%)	28 (56.00%)	23 (46.00%)
χ^2	5.459	4.154	4.348	4.199
P	0.019	0.042	0.037	0.040

Note: Angina pectoris: cases of angina pectoris symptoms improvement; TCM: cases of TCM symptoms improvement; ECG: cases of ECG improvement; Nitroglycerin stopping and decrease: cases of nitroglycerin stopping and decrease.

anticoagulation was performed using EDTA. The nucleated cells were separated by lymphocyte separation solution and the total RNA was extracted using the high purity total RNA rapid extraction kit (Sigma company); 2) cDNA was synthesized by the reverse transcription of mRNA using first strand cdna synthesis kit (Sigma company); 3) the design and synthesis of primers: according to human 5-LOX, GAPDH gene sequence in Genbank, primers were designed by Primer 5.1 software and synthesized by the sigma company and the length of 5-LOX primer amplified fragment was 170 bp, the sense strand was 5'-TGACCACGGAGATGGTAGAGTG-3' and the antisense strand was 5'-ATACAGCAAGCAGATGGGAGC-3', while the length of internal reference GAPDH amplified fragment was 258 bp, the sense strand was 5-AGAAGGCTGGGGCTCATTTG-3', the antisense strand was 5'-AGGGGCCATCCACAGTCTTC-3'; 4) PCR amplification: the reaction was carried out using a full automatic fluorescence quantitative PCR instrument (CFX96 Touch™ Real-Time PCR Detection System). There were 0.4 μL upstream and downstream primers respectively, real time quantitative PCR reaction premix (2*) 10.0 μL and cDNA 2.0 μL (final concentration: 0.2 μmol/L) in the 20 μL reaction system; 5) amplification conditions: 95.0°C initial denaturation for 3 min, 95.0°C denaturation for 10 s, 61.0°C annealing/extension for 20 s, a total of 40 cycles, the solubility curve conditions were: at 70°C to 95°C, it increased 0.5°C per 10 s and the amplified products were analyzed and sequenced by solubility curve to determine product specificity; 6) results analysis: the automatic analysis of results was performed by PCR special analysis software BIO-RAD CFX Manager, the cycle threshold was negatively correlated with the initial expression quantity of mRNA, ΔCt value represents the expression, the target gene relative to the internal reference gene, $\Delta Ct=Ct_{5\text{-}LOX}-Ct_{GAPDH}$, meanwhile $2^{-\Delta\Delta Ct}$ formula was used for relative quantification.

Statistical method

The data analysis was performed by statistical software SPSS17.0 and the measurement data was expressed by mean ± sd. The three groups of sample measurement data was measured by F test. The two independent sample measurement data was detected using t test. The enumeration data was expressed as a ratio, and measured by χ^2 test. The total ion current obtained by Q1 scanning was performed with data processing by the MZmine software, and then the processed data were then introduced into SIMCA-P software (version 11.5, Umetricsa AB, Umea, Sweden) for the principal component analysis. Contrast analysis of patients both in UA and C group was finished using PLS-DA, and the comparison between two groups was carried out by t test.

Results

Comparison of treatment for UA between M group and N group

Compared with N group, there was significant increase in the improvement rate of angina pectoris symptoms (P=0.019), that of TCM symptoms (P=0.042), that of ECG (P=0.037) and that of the stopping and decrease rate of nitroglycerin (P=0.040). The differences were statistically significant (see **Table 2**).

PLS-DA analysis of the treatment group and the C group

After the data processed by MZmine was introduced into the software SIMCA-P11.5, the project was set up and work set was created. The data in treatment group and the C group were expressed as jb and jk respectively, and the PLS-DA model was assigned for matching [12]. The fitting analysis found that there were significant differences between the two groups of samples and found specific indicators might cause these differences through a large num-

Table 3. Analysis of data sheets

ID	M/Z	RT	t	*P* value	Pls-da vip
11 910	374.45	8.44	5.565	>0.05	2.17
11 922	358.41	9.48	5.530	>0.05	2.15
11 869	330.46	8.34	5.508	>0.05	2.17
11 854	302.35	7.50	5.469	>0.05	2.10
7 478	633.45	15.89	5.459	>0.05	-
11 914	374.28	8.51	5.355	>0.05	2.03
7 425	466.45	12.55	5.324	>0.05	2.65
5	377.44	12.50	5.323	>0.05	2.03
1 374	512.22	11.6	5.293	>0.05	2.33
11 800	357.35	8.54	5.238	>0.05	2.23

Table 4. Potential marker screening

ID	Peak height in d VS m	NAME
7970	↑	10, 11-dihydro-LTB4
		6, 7-dihydro-12-epi-LTB4

ber of data analysis. After variance analysis, the data analyzed by PLS-DA was detected by t test [13]. Then, the SD, V and CV of two groups were calculated. The indexes of V=0 in two groups were deleted, and we found there was nothing in the health C group after the indexes of V_D>0 and V_M=0 were screened; the indexes produced in the UA group were qualitative changes, not requiring the use of the t test. The indexes of V_D>0 and V_M>0 were further screened, the homogeneity test of variance was used to estimate $0.4<CV_D/CV_M<2.5$ and meanwhile $CV_D<1.5$ & $CV_M<1.5$ (see **Table 3**).

Analysis of potential markers

MS/MS analysis was performed on the samples with higher relative content in all samples from the collected data sheets. Possible potential markers were screened through the MS/MS search library and MZmine search library, and significant changes of LTB4 were showed (see **Table 4**) [14].

Comparison of the contents of LTB4 in peripheral blood of three groups of patients

ELISA results showed that the content of LTB4 in patients' peripheral blood of M group (P<0.001), C group (P<0.001) were both lower than that of N group. The differences were statistically significant (as shown in **Figure 1**).

Comparison of the expression levels of 5-LOX mRNA in patients' peripheral blood of three groups

RT-PCR results showed that the expression levels of 5-LOX mRNA in patients' peripheral blood of M group (P=0.041), C group (P= 0.012) were both lower than that of N group. The differences were statistically significant (as shown in **Figure 2**).

Discussion

UA is common in adults who aged over 40. It will develop rapidly after the age of 50. The age analysis of this study showed that the patients' ages mainly concentrated on the age between 50 and 70. For the elderly debilitated patients, their functional decline of five viscera leaded to the qi deficiency of the five viscera. After a long period of various treatments, people's vital qi would become weaker. Such a vicious cycle would eventually develop into serious illness such as myocardial infarction and it would even threaten life. The development of TCM took the clinical curative effect as the core, syndrome differentiation and treatment and holistic concept as the theories and the thinking characteristics. The core foundation of TCM's understandings on disease, clinical diagnosis and curative effect judgments was syndrome. Through a great deal of clinical practice, it has proved that TCM syndrome differentiation and treatment had good curative effects and obvious advantages.

Yang xin tang originated from *Syndrome treatment criterion·miscellaneous diseases kind prescription* written by Wang Kentang of Ming Dynasty. The composition prescription which composed of nourishing heart and tranquilizing drugs and invigorating qi and invigorate blood drugs was its distinctive feature. It mainly treated little blood deficiency, terrified restless. We found that yang xin tang could not only alleviate the pain of patients, but also reduce the morbidity of the disease and reduce the dosage of nitroglycerin in clinical trials [15]. The results of this experiment showed that the improvement of ECG results and the increase of the reduce number of nitroglycerin stop could be realized by yang xin tang. It confirmed the effectiveness of yang xin tang in the treatment of UA. The ECG of the study showed that S-T segment depression and T wave inversion

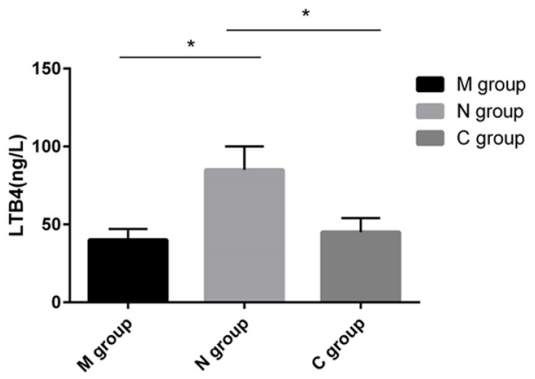

Figure 1. Comparison of the contents of LTB4 in peripheral blood of three groups of patients. Compared with N group, *P<0.05.

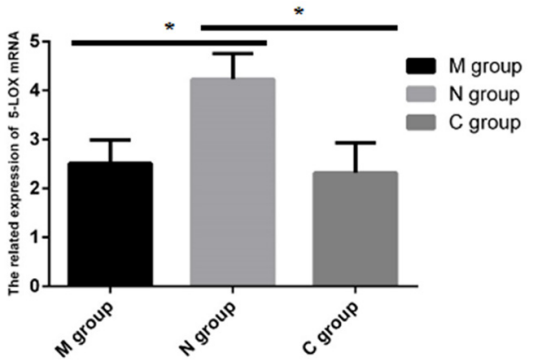

Figure 2. Comparison of the expression levels of 5-LOX mRNA in patients' peripheral blood of three groups. Compared with N group, *P<0.05.

were markedly improved after patients using yang xin tang. This indicated that yang xin tang could improve the degree of myocardial ischemia.

Because the clinical angina pectoris is not only related to unstable plaque secondary thrombosis, but also associated with coronary artery spasm. Sensitive part of the coronary artery spasm is due to endothelial function disorders under pathological conditions, elevated endothelin, the decline of bioavailability which is caused by impaired nitric oxide synthase function, the increase of oxygen free radical in blood, thus leading to acute vascular stress and so on [16]. We speculated that some patients' angina pectoris were mainly induced by coronary artery spasm, so when the condition was relieved, ECG had an obvious improvement [17]. At the same time, this study chose symptomatic improvement of TCM symptoms and angina pectoris symptoms to assess the influence of antianginal drugs treatment plan on quality of life of patients with stable angina pectoris. It was confirmed by the results that yang xin tang could significantly improve the patients' symptoms of angina pectoris and TCM symptoms. The research on reduce rate of nitroglycerin stop showed that yang xin tang could significantly reduce patients' use of nitroglycerin. Nitroglycerin is one of the most widely used drugs to treat angina pectoris. It made up decreased nitric oxide which was caused by endothelial injury through releasing exogenous nitric oxide, in order to play the role of relaxing vascular smooth muscle [18-20].

Some studies have pointed out that yang xin tang repaired damaged vascular endothelium by adjusting disordered endothelial function, so that restored the body's releasing ability. There were essential differences between exogenous release of nitroglycerin and its mechanism [21]. We could not ignore the drug resistance of nitroglycerin in clinically. At present, the effective way to reduce its drug resistance is to reduce the dosage of nitroglycerin [22]. As how to prevent or reduce the drug resistance of nitroglycerin, this study draw the conclusion that yang xin tang could significantly reduce the use of nitroglycerin in patients, and this conclusion had practical significance. The results also provide a clear clinical basis for drug combination of yang xin tang and nitroglycerin.

Metabolomics is an emerging science which studies organism's endogenous metabolites quantity, variety and its change rules [23]. It mainly studies the endogenous metabolites of the whole organism, the systemic organ and its internal and external environment. It is an important component of systems biology. It provides new ideas and methods for the diagnosis and treatment of diseases and TCM syndrome research [24]. This study used ideas and methods of metabolomics research for reference. Obstruction of the heart qi deficiency syndrome was seen as a starting point. The research showed that through doing full spectrum analysis for the serum metabolic group of patients with UA pectoris and healthy volunteers, abnormal elevation of LTB4 was found in the plasma of patients. It confirmed that there were inflammatory responses in UA patients' coronary artery endothelium. It would trigger

coronary artery spasm or form unstable atherosclerotic plaque, eventually led to recurrent angina pectoris [25].

5-LOX is a member of the lipoxygenase family, which is a key enzyme to catalyze the conversion of arachidonic acid to leukotriene [26, 27]. In this study, we found that 5-LOX significantly increased in the blood of patients with UA and the content of LTs subtype TLB4 in serum was significantly increased. And through the experiment, we found that yang xin tang could reduce the production of 5-LOX and the content of TLB4 in serum. Through the above results, it was confirmed that 5-LOX was involved in the metabolic pathway of leukotriene B4. Yang xin tang might improve the symptoms of patients with UA by reducing the production of 5-LOX and played an important role in patient's outcome process. However, this study has not yet fully confirmed that yang xin tang achieved the mechanism of heart protection through the 5-LOX/LTB4 pathway, the relevant basic experiments should be further carried out: First, to determine that yang xin tang did reduce the expression of 5-LOX, while reducing LTB4 content, improving the disease status of UA mice and reducing the degree of myocardial injury; second, to confirm that after the use of 5-LOX inhibitor, LTB4 content decreased, UA mice disease status improved, myocardial damage decreased; third, to verify that LTB4 antagonist improved the outcome of UA mouse disease.

In summary, the adverse reaction induced by UA could significantly improve by yang xin tang. Its possible mechanism is to treat UA by exerting an influence on the metabolic pathway of 5-LOX and then reducing the production of LTB4, so as to have therapeutic effects on UA. However, due to the relative shortage of the sample capacity, it might have unavoidable error. In order to further prove the therapeutic effects of yang xin tang on UA, in the future research we will increase the sample capacity and improve clinical design, such as double blind, placebo control and so on.

Acknowledgements

This paper was supported by the Doctoral Innovation Funds of Heilongjiang University of Chinese Medicine (2015bs07).

Disclosure of conflict of interest

None.

Address correspondence to: Jing Sun and Yabin Zhou, Heilongjiang University of Chinese Medicine, No.24 Heping Road, Xiangfang District, Harbin 150040, Heilongjiang Province, China. Tel: +86-0451-82193000; E-mail: sunjing101000@126.com (JS); zhouyabin101000@126.com (YBZ)

References

[1] Wang L, Mao S, Qi JY, Ren Y, Guo XF, Chen KJ and Zhang MZ. Effect of danlou tablet () on peri-procedural myocardial injury among patients undergoing percutaneous coronary intervention for non-ST elevation acute coronary syndrome: a study protocol of a multicenter, randomized, controlled trial. Chin J Integr Med 2015; 21: 662-666.

[2] Fanning JP, Nyong J, Scott IA, Aroney CN and Walters DL. Routine invasive strategies versus selective invasive strategies for unstable angina and non-ST elevation myocardial infarction in the stent era. Cochrane Database Syst Rev 2016; Cd004815.

[3] Ostovaneh MR, Poustchi H, Hemming K, Marjani H, Pourshams A, Nateghi A, Majed M, Navabakhsh B, Khoshnia M, Jaafari E, Mohammadifard N, Malekzadeh F, Merat S, Sadeghi M, Naemi M, Etemadi A, Thomas GN, Sarrafzadegan N, Cheng KK, Marshall T and Malekzadeh R. Polypill for the prevention of cardiovascular disease (polyIran): study design and rationale for a pragmatic cluster randomized controlled trial. Eur J Prev Cardiol 2015; 22: 1609-1617.

[4] Wang WL, FU CW, She H. Effect of yangxinyigan decoction on the content of TXB_2 and 6-keto-PGF_(1α) in blood plasma of patients with ischemic apoplexy sequelae. Journal of Emergency in Traditional Chinese Medicine 2008; 17: 895-897.

[5] Liu YZ, Zhou YB, Pan XB. The effect of Yangxin decoction on plasma TXB_2 content and the tunica intima thickness in rabbits with myocardial ischemia. Chinese Journal of Integrative Medicine on Cardio-/Cerebrovascular Disease 2006; 4: 1067-1068.

[6] Liu LH. Report of unstable angina with coronary artery disease treated by yangxintongmai decoction. Hebei Journal of Traditional Chinese Medicine 2008; 30: 800-802.

[7] Liu YZ, Zhou YB, Han JR, Pan XB. Effect of Yangxin decoction on NO and NOS content in rabbit with unstable angina. Chinese Journal

of Integrative Medicine on Cardio-/Cerebrovascular Disease 2006; 4: 978-979.
[8] Haïat R. Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the joint international society and federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature. Circulation 1979; 59: 607-609.
[9] Zheng XY. Guiding principle of clinical research on new drugs of Traditional Chinese Medicine (Trial). 2002.
[10] Shi ZX and Gu WL. [Exploration of TCM syndrome differentiation of coronary heart disease and coronary arteriography]. Zhongguo Zhong Xi Yi Jie He Za Zhi 2007; 27: 76-79.
[11] Kobuchi S, Matsuno M, Fukuda E, Ito Y and Sakaeda T. Development and validation of an LC-MS/MS method for the determination of tofogliflozin in plasma and its application to a pharmacokinetic study in rats. J Chromatogr B Analyt Technol Biomed Life Sci 2016; 1027: 227-233.
[12] Gromski PS, Muhamadali H, Ellis DI, Xu Y, Correa E, Turner ML and Goodacre R. A tutorial review: metabolomics and partial least squares-discriminant analysis--a marriage of convenience or a shotgun wedding. Anal Chim Acta 2015; 879: 10-23.
[13] Wu X, Zhu JC, Zhang Y, Li WM, Rong XL and Feng YF. Lipidomics study of plasma phospholipid metabolism in early type 2 diabetes rats with ancient prescription Huang-Qi-San intervention by UPLC/Q-TOF-MS and correlation coefficient. Chem Biol Interact 2016; 256: 71-84.
[14] Kosjek T, Negreira N, Heath E, de Alda ML and Barceló D. Biodegradability of the anticancer drug etoposide and identification of the transformation products. Environ Sci Pollut Res Int 2016; 23: 14706-14717.
[15] Kosjek T, Negreira N, Heath E, de Alda ML and Barceló D. Biodegradability of the anticancer drug etoposide and identification of the transformation products. Environ Sci Pollut Res Int 2016; 23: 14706-14717.
[16] Ong P, Aziz A, Hansen HS, Prescott E, Athanasiadis A and Sechtem U. Structural and functional coronary artery abnormalities in patients with vasospastic angina pectoris. Circ J 2015; 79: 1431-1438.
[17] Lyzohub VH, Artemchuk OO, Briuzhina TS, Voloshyna OO and Nyzhenkovs'kyĭ OI. [Efficiency of early atorvastatine treatment in patients with unstable angina]. Lik Sprava 2007; 18-22.
[18] Steinhorn BS, Loscalzo J and Michel T. Nitroglycerin and nitric oxide--a rondo of themes in cardiovascular therapeutics. N Engl J Med 2015; 373: 277-280.
[19] Mao M, Sudhahar V, Ansenberger-Fricano K, Fernandes DC, Tanaka LY, Fukai T, Laurindo FR, Mason RP, Vasquez-Vivar J, Minshall RD, Stadler K and Bonini MG. Nitroglycerin drives endothelial nitric oxide synthase activation via the phosphatidylinositol 3-kinase/protein kinase B pathway. Free Radic Biol Med 2012; 52: 427-435.
[20] Opelt M, Eroglu E, Waldeck-Weiermair M, Russwurm M, Koesling D, Malli R, Graier WF, Fassett JT, Schrammel A and Mayer B. Formation of nitric oxide by aldehyde dehydrogenase-2 is necessary and sufficient for vascular bioactivation of nitroglycerin. J Biol Chem 2016; 291: 24076-24084.
[21] Xu JM, Li YQ, Liu YE, Zhang XM, Zhao R, Zhou YW. Protective effect of Yangxin granules on endothelial cells and myocardium in mongrel dogs with myocardial infarction. Chinese Journal of Biochemical Pharmaceutics 2007; 28: 30-33.
[22] Tvedskov JF, Iversen HK, Olesen J and Tfelt-Hansen P. Nitroglycerin provocation in normal subjects is not a useful human migraine model? Cephalalgia 2010; 30: 928-932.
[23] Liu XJ, Locasale JW. Metabolomics: a primer. Trends in Biochemical Science 2017; 1-11.
[24] Liesenfeld DB, Grapov D, Fahrmann JF, Salou M, Scherer D, Toth R, Habermann N, Bohm J, Schrotz-King P, Gigic B, Schneider M, Ulrich A, Herpel E, Schirmacher P, Fiehn O, Lampe JW and Ulrich CM. Metabolomics and transcriptomics identify pathway differences between visceral and subcutaneous adipose tissue in colorectal cancer patients: the ColoCare study. Am J Clin Nutr 2015; 102: 433-443.
[25] Yasue H, Nakagawa H, Itoh T, Harada E and Mizuno Y. Coronary artery spasm--clinical features, diagnosis, pathogenesis, and treatment. J Cardiol 2008; 51: 2-17.
[26] Heemskerk MM, Giera M, Bouazzaoui FE, Lips MA, Pijl H, van Dijk KW and van Harmelen V. Increased PUFA content and 5-lipoxygenase pathway expression are associated with subcutaneous adipose tissue inflammation in obese women with type 2 diabetes. Nutrients 2015; 7: 7676-7690.
[27] Ziboh VA, Naguwa S, Vang K, Wineinger J, Morrissey BM, Watnik M and Gershwin ME. Suppression of leukotriene B4 generation by ex-vivo neutrophils isolated from asthma patients on dietary supplementation with gammalinolenic acid-containing borage oil: possible implication in asthma. Clin Dev Immunol 2004; 11: 13-21.