

Clinical Observations

Effects of Liandou Qingmai Recipe (连豆清脉方) on Endothelin-1, Nitric Oxide, Interleukin-6 and Interleukin-10 Levels in Patients with Coronary Heart Disease

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Objective: To observe effects of Liandou Qingmai Recipe (连豆清脉方) on endothelin-1 (ET-1), nitric oxide (NO), interleukin-6 (IL-6) and IL-10 levels in patients with coronary heart disease.

Methods: Total 101 cases with coronary heart disease were randomly divided into a treatment group ($n=45$) treated by Liandou Qingmai Recipe and a standard treatment group (control group, $n=56$), with a normal group of 16 health persons set up. Changes of ET-1, NO, IL-6 and IL-10 levels were measured before treatment and after treatment for two weeks. And the data were analyzed by SPSS 16.0 statistic software.

Results: Before treatment, the levels of ET-1, IL-6 and IL-10 levels were significantly higher and NO was significantly lower in the patients with coronary heart disease than those in the normal group (90.7 ± 12.7 ng/L vs 41.8 ± 13.5 ng/L, 9.17 ± 0.18 ng/L vs 1.10 ± 0.08 ng/L, 1.94 ± 0.26 ng/L vs 1.09 ± 0.06 ng/L, and 92.2 ± 17.7 μ mol/L vs 124.5 ± 27.2 μ mol/L; all $P<0.05$), with no significant differences in the levels of ET-1, NO, IL-6 and IL-10 between the treatment group and the control group ($P>0.05$); After treatment, ET-1 and IL-6 significantly decreased in the treatment group and the control group, and NO increased in the treatment group; And IL-6 level was significantly lower and NO level was higher in the treatment group than those in the control group (4.48 ± 1.22 ng/L vs 5.13 ± 1.85 ng/L, 117.4 ± 22.3 μ mol/L vs 92.4 ± 17.1 μ mol/L; both $P<0.05$); There was a positive correlation between IL-6 and IL-10, and a negative correlation between NO and IL-10 ($r=0.142$, $r=-0.152$; both $P<0.05$).

Conclusion: Liandou Qingmai Recipe can decline IL-6, IL-10 and ET-1 levels, and raise NO level in patients with coronary heart disease on the basis of standard treatment, so as to inhibit endothelial inflammatory response, improve vascular endothelial function, with stronger anti-AS action; And vascular endothelial lesion is related with inflammatory response.

Keywords: coronary heart disease; Liandou Qingmai Recipe (连豆清脉方); endothelin-1; interleukin-6; interleukin-10; nitric oxide

Atherosclerosis (AS) is pathological basis of coronary heart disease and its basic pathogenesis is deficiency in origin and excess in superficiality, and phlegm and blood stasis. The authors hold that AS is caused by pathogenic heat, phlegm and blood stasis jointly transferring into turbid due to insufficiency of kidney, and “heat, turbid and deficiency of kidney” are the most important pathogenesis.^{1,2} Therefore, “clearing away heat, removing the turbid and tonifying the kidney” is basic principle for treatment of AS.³ On these grounds, Liandou Qingmai Recipe (连豆清脉方) the authors developed has better clinical effects in prevention and treatment of AS and relative diseases. A study has confirmed that this recipe can further decrease brachial-ankle pulse wave velocity and increase ankle-brachial index in atherosclerosis patients on the basis of standard treatment.⁴ It has a better effect of improving arterial rigidity degree, and it is verified that the recipe can decrease low density lipoprotein-cholesterol level and atherosclerosis-inducing index in plasma in ather-

osclerosis patients, giving full play to anti-AS; At the same time, it can decrease ultra-sensitive C-response protein and peripheral white blood cells, with a better anti-inflammation action. Thus, it is inferred that the mechanism of Liandou Qingmai Recipe for treatment of AS is possibly involved in anti-inflammation and improvement of endothelial dysfunction. So, in the study, effects of Liandou Qingmai Recipe on endothelin-1 (ET-1), nitric oxide (NO), interleukin-6 (IL-6), and IL-10 in atherosclerosis patients were investigated. A report is following.

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METHODS

Diagnostic Criteria of Coronary Heart Disease

Diagnosis of coronary heart disease referred to *The Nomenclature and Diagnostic Criteria of Ischemic Heart Diseases* issued by WHO.⁵

General Data

Altogether, 101 patients of coronary heart disease and 16 health volunteers were included in this study. The patients all were inpatients in the Heart Internal Medicine Department, Wuxi Hospital Affiliated to Nanjing University of Traditional Chinese Medicine from June 2007 to December 2009. They were randomly divided into Liandou Qingmai Recipe treatment group (treatment group) and standard treatment group (control group) according to random number table. In the treatment group of 45 cases, 20 cases were male and 25 cases were female; Aged 51-87 years, averaging (71.2 ± 8.4) years; The course of disease was 1 h to 40 years with a mean of (median 5.27 (1.00-12.02)) years. In the control group of 56 cases, 24 cases were male and 32 cases were female; Aged 54-86 years with a mean of (70.4 ± 8.9) years; The course of disease was 1 h to 40 years averaging (median 5.14 (1.00-11.97)) years. There were no statistically significant differences between the two groups in age, sex and course of disease. Members of the normal control group (normal group) of 16 healthy volunteers were staff and workers at their posts or retirees in this hospital after matching age and sex.. This study through the hospital's ethics committee for examination and approval, all study subjects are informed consent and signed informed consent.

Criteria of Inclusion

Meet the diagnostic criteria of coronary heart disease and conform to any one item of following conditions: remote myocardial infarction, hemodynamically stable acute myocardial infarction or angina pectoris, positive sports plate test with clinical symptom and sign, at least one blood vessel or at least one part of a vessel narrowing over 60% found by coronary arteriography; and the patients voluntarily signed the a fact-known consent.

Criteria of Exclusion

The patients of insufficiency of the spleen-yang in traditional Chinese medicine, hemodynamically unstable angina pectoris and acute myocardial infarction, severe arrhythmia, complicated other severe heart diseases, severe dysfunction of liver and kidney, severe original diseases of lung and brain, psychosis, or refusing to sign a fact-known consent, were excluded.

Treatment Methods

The patients in the treatment group and the control group were treated with a standard treatment, including nitrate type drugs (such as Isosorbide mononitrate tablets 20 mg,

twice each day, Isosorbide dinitrate, 5-10 mg, thrice daily, and nitroglycerin according to patient's condition), Aspirin (100-300 mg, once each day), Statins type drugs for regulating lipids (such as Atovastatin calcium tablets 10-20 mg, once each day, Fluovastatin sodium capsules 40 mg, once each night, etc.), Angiotensin-converting enzyme inhibitor (Benazepril tablets 10 mg, once each day, Pediopril tablets 4 mg, once daily, etc); For the patient of no contraindication, β -receptor blockers, etc. (Metoprolol tablets 6.25 mg-100 mg, twice each day, Bisoprolol tablets 2.5 mg-5.0 mg, once each day, etc.) were used for long term in whole course; There were no statistically significant differences between the two group in the drug kinds, dose and therapeutic course for the standard treatment. For the patients in the treatment group, Liandou Qingmai Recipe composed of Lianqiao (Fructus Forsythiae) 15 g, Huanglian (Rhizoma Coptidis) 3 g, Yeliaodou (野料豆) 15 g, Chishao (Radix Paeoniae) 10 g, Laifuzi (Semen Raphani) 10 g, and so on, was added on the basis of standard treatment, which prepared by Pharmaceutics Department in the hospital and the herbs were purchased from Jiangzhong Pharmaceutical Factory, 250 mL each decoction, oral administration, twice each day, two weeks consisted of one therapeutic course.

Collection and Preservation of Samples

Six mL blood was taken from vein of the patient within 24 h after hospitalization on empty stomach in the morning. Among them, 2 mL was slowly poured into a tube with 30 μ L 10% EDTA and mixed, and stood for 1 h at room temperature, and then centrifuged at 3000 r/min for 10 min. The supernatant was taken and poured into a tube, sealed and kept at -70 °C for ET-1 determination; Other 4 mL blood were poured into a dry tube and stood for 1 h at room temperature, and then centrifuged for 10 min at 3000 r/min. A part of the supernatant was kept in a tube and sealed, and kept at -70 °C for NO determination; And another part of the supernatant was used for determination of IL-6 and IL-10 determination immediately.

After treatment for two weeks, collection, preservation and determination of the above samples were repeated. ET-1 and NO were determined together once each 6 months.

Observation Indexes and Determination Methods

NO kit purchased from Nanjing Jiancheng Bioengineering Institute was used for determination of NO with 751G spectrophotometer; ET-1 radioimmunoassay kit made by Beijing North Biotechnology Institute was used for automatic determination of ET-1 with GC911 radioimmunoanalyser; IL-6 and IL-10 were determined with special agents by IMMULITE 1000 full-automatic chemiluminescence immunoanalyser (Siemens Co., Germany).

Statistical Analysis

The data were statistically analyzed with SPSS16.0 software packet. The measurement data were expressed as $\bar{x} \pm s$; Two-side student-*t* test of two independent samples was used for comparison of therapeutic effects between groups, and two-side student-*t* test of paired samples was used for comparison of therapeutic effects before and after treatment in-group, significant level $\alpha=0.05$.

RESULTS

Comparison of IL-6, IL-10, ET-1 and NO Levels before Treatment between the Coronary Heart Disease Group and the Normal Group

Before treatment, ET-1, IL-6, IL-10 and NO levels were 90.7 ± 12.7 ng/L, 9.17 ± 0.18 ng/L, 1.94 ± 0.26 ng/L and 92.2 ± 17.7 μ mol/L in the coronary heart disease group (the control group plus the treatment group), and 41.8 ± 13.5 ng/L, 1.10 ± 0.08 ng/L, 1.09 ± 0.06 ng/L, and 124.5 ± 27.2 μ mol/L in the normal group, respectively. ET-1, IL-6, IL-10 levels before treatment in the coronary heart disease group were significantly higher and NO was lower than those in the normal group (all $P < 0.05$, Table 1).

Effects of Liandou Qingmai Recipe on IL-6, IL-10, ET-1 and NO levels

Before treatment, there were no significant differences in IL-6, IL-10, ET-1 and NO levels between the treatment group and the control group ($P > 0.05$). After treatment, ET-1 and IL-6 levels significantly decreased ($P < 0.05$) and NO and IL-10 did not significantly change in the control group; ET-1 and IL-6 levels decreased and NO increased significantly (both $P < 0.05$), and IL-10 did not significantly change in the treatment group; NO level was significantly higher and IL-6 was significantly lower in the treatment group than those in the control group (both $P < 0.05$); And there were no significant differences in ET-1 and IL-10 levels between the two groups (Table 1).

Correlativity between Endothelial Function and Inflammatory Indexes

There was a positive correlation between IL-6 and IL-10 ($r=0.142$, $P=0.039$) and a negative correlation between NO and IL-10 ($r=-0.152$, $P=0.027$). There was no statistically correlation between ET-1 and NO, NO and IL-6, ET-1 and IL-6, ET-1 and IL-10.

Table 1. Effects of Liandou Qingmai Recipe (连豆清脉方) on IL-6, IL-10, ET-1 and NO levels

| Group | Cases | IL-6 (ng/L) | IL-10 (ng/L) | ET-1 (ng/L) | NO (μ mol/L) |
|------------------------------|-------|-----------------------------------|-----------------|------------------|------------------------------------|
| Normal group | 16 | 1.10 ± 0.08 | 1.09 ± 0.06 | 41.8 ± 13.5 | 124.5 ± 27.2 |
| Control group ¹ | | | | | |
| Before treatment | 56 | 9.22 ± 2.05 | 1.95 ± 0.32 | 89.9 ± 13.1 | 91.9 ± 18.2 |
| After treatment | | $5.13 \pm 1.85^*$ | 1.65 ± 0.18 | $64.6 \pm 9.8^*$ | 92.4 ± 17.1 |
| Treatment group ² | | | | | |
| Before treatment | 45 | 9.11 ± 1.36 | 1.93 ± 0.19 | 91.6 ± 11.9 | 92.5 ± 17.7 |
| After treatment | | $4.48 \pm 1.22^{*\blacktriangle}$ | 1.82 ± 0.19 | $59.5 \pm 8.7^*$ | $117.4 \pm 22.3^{*\blacktriangle}$ |

Notes: Compared with the data before treatment, $^*P < 0.05$; Compared with the control group, $^{*\blacktriangle}P < 0.05$.

1. Control (standard treatment group); 2. Treatment (Liandou Qingmai Recipe treatment group)

DISCUSSION

Atherosclerosis is physiopathologic basis of coronary heart disease, and it has endothelial dysfunction at various stages from its formation and development to occurrence of clinical symptoms and signs. Vascular active substances secreted from vascular endothelium play an important metabolic and regulatory role. Among them, endothelins (ETs) are the most important vasoconstrictive substance, and it plays an important role in the course of atherosclerosis. It can induce contraction of various vessels with the most sensitive for coronary artery. ET has 3 kinds of isomer and ET-1 has the strongest vasoconstrictive action. In vitro experiment indicates that the vasoconstrictive action of ET-1 is 10 times stronger than angiotensin II, and it is the strongest vasoconstrictive factor known at present.⁶ While, NO was a gas molecule with varied biological activities, and with very short half-life period, being unstable, and it has been proved that it is diastolic factor derived from

endothelium with actions of strongly dilating blood vessels, inhibiting aggregation and activation of blood platelet, inhibiting infiltration and absorption of neutrophilic granulocytes and mononuclear leukocytes for vascular wall, inhibiting proliferation of vascular smooth muscle and anti-thrombosis. In atherosclerosis, lesion of endothelial function induces decrease of NO synthesis and increase of NO decomposition, leading to decrease of NO level, disturbance of fibrinolytic function and reduction of anti-thrombosis, further deteriorating atherosclerosis.⁷ This study confirmed that in the patients with coronary heart disease ET-1 level was significantly higher and NO significantly was lower than those in the healthy persons, which are uniform with literature and further verify that disturbance of endothelial function is an important pathological basis of AS.

Liandou Qingmai Recipe is composed mainly of Lianqiao (Fructus Forsythiae), Huanglian (Rhizoma

Coptidis), Yeliaodou (野料豆), Chishao (Radix Paeoniae), Laifuzi (Semen Raphani), and so on. Modern pharmacological studies find that these Chinese medicines have varied pharmacologic functions, such as antisepsis, anti-inflammation, decreasing blood pressure, reducing blood lipids, and antioxidation, and so on. Lizuka T, et al. found that forsythiaside extracted and separated from fruits of *Forsythia suspensa* had antagonistic action on calcium ion.⁸ These pharmacological mechanisms are beneficial for improving arterial endothelial function. This study further verified that on the basis of standard treatment, Liandou Qingmai Recipe could decrease ET-1 concentration, alleviate the unfavorable vasoconstrictive action of ET-1 on coronary heart disease; increase NO level, strengthening the actions of dilating blood vessels, inhibiting aggregation and activation of blood platelets, inhibiting infiltration and absorption of neutrophilic granulocytes and mononuclear leukocytes for vascular wall, inhibiting proliferation of vascular smooth muscle cells and anti-thrombosis in the patient with coronary heart disease, so as to improve endothelial function.

Inflammatory response is an important mechanism for occurrence and development of AS and coronary heart disease. Among them, IL-6 is one of strong inflammatory factors inducing inflammatory response, and it makes generation of vascular endothelial active oxygen cluster increase, oxygen free radicals evoking oxidative stress, inducing dysfunction of vascular endothelium, accelerating atherosclerosis.⁹ IL-6 expression is found in both the part of plaque of coronary atherosclerosis and the arterial wall injured by atherosclerosis, and the expression amount is 10–40 times higher than that in the normal tissue.¹⁰ Plasma IL-6 concentration in the patient of unstable angina pectoris is significantly higher than that in the patients of stable angina pectoris or the normal control group.¹¹ IL-10 is a main anti-inflammatory cytokine, and it can inhibit activities of mononuclear cells and macrophages, etc., and down-regulate the stimulating action of chemotactic factor and inflammatory factor on proliferation of smooth muscular cells, and directly inhibit expression of adhesive molecules, prevent ischemia / reperfusion lesion of myocardium, protect myocardium. IL-10 also can reduce metalloprotease activity in the plaque.¹² Smith, et al. found that serum IL-10 concentration in the patient of unstable angina pectoris was significantly lower than that in the patient of stable angina pectoris.¹³ It is indicated that IL-10 is inversely proportional to AS plaque unstable degrees. Therefore, IL-10 plays possibly an important role in inhibiting occurrence and development of coronary heart disease. In this study, it also was proved that IL-6 level in the patient of coronary heart disease was significantly higher than that in the patients of the normal group, indicating that endothelial function lesion is related with inflammatory response.

Antibacterial action of Huanglian (Rhizoma Coptidis) and Lianqiao (Fructus Forsythiaein), etc. in the Liandou Qingmai Recipe is well known clinically. A study proved that extract of Huanglian (Rhizoma Coptidis) can reduce breaking times of aortic atherosclerotic plaque in ApoE-gene knockout mice, helping stabilize vulnerable atherosclerotic plaque.¹⁴ While this present study proved that on the basis of standard treatment, Liandou Qingmai Recipe could decrease IL-6 and IL-10 concentrations, inhibit injuring action of IL-6 on vascular endothelium in the patient of coronary heart disease, so as to improve vascular endothelial function. IL-6 and ET-1 were decreased and NO was increased in both the treatment group and the control group, but after combination of Liandou Qingmai Recipe with standard treatment, the effects of decreasing ET-1 and increasing NO were significantly strengthened. Therefore, Liandou Qingmai Recipe can further strengthen the effects of inhibiting vascular endothelial inflammatory response, inhibiting release of vascular endothelins, promoting release of NO, so as to improve vascular endothelial function and artherosclerosis.

Also, a study found that compared with the patients of no occurrence of new coronary arterial event, the ratio of IL-6 to IL10 significantly increased in the patients of occurrence of new coronary arterial event.¹⁵ It is inferred that IL-10 and IL-6 ought to show antagonistic relation. But, in both the control group and the treatment group, both IL-6 and IL-10 decreased, so they could not play anti-inflammatory role in the two groups through increasing IL-10 concentration. Further analysis found that IL-6 and IL-10 were positive correlation ($r=0.142$). Thus, the authors hold that IL-10 possibly does not have actively inhibiting action on AS, while at the unstable stress state of AS plaque, IL-10 passively increases the inhibiting effect on inflammatory response, so as to inhibit advance of AS.

In addition, in the patient of coronary heart disease plasma ET level increased, NO level decreased, with a negative correlation between them. Zellers, et al.¹⁶ found that in normal physiologic condition, ET can stimulate release of endothelial NO, so as to decrease itself vasoconstrictive action; ET and NO restrict, antagonize and coordinate each other, being a dynamic balance relation. In this study, there was no a statistically significant negative correlation between ET-1 and NO. Theoretically, the stronger the inflammatory response, the more severe the endothelial lesion, thus inflammatory response indexes and endothelial function indexes should have certain correlativity. This hypothesis was partially confirmed by this study: NO and IL-10 showed a negative correlation ($r=0.152$). This conforms with that the physiopathologic mechanisms, i.e., when inflammatory response is severe and IL-10 increases, endothelial function will be injured and NO release will reduce; when inflammatory response is inhibited and

IL-10 decreases, endothelial function will improve and release of NO will increase.

In brief, on the basis of standard treatment, Liandou Qingmai Recipe can decrease IL-6, IL-10 and ET-1 levels and increase NO level in the patient with coronary heart disease, so as to inhibit the endothelial inflammatory response, improve vascular endothelial function, with stronger anti-AS action. And the data in the study proves partially correlativity between inflammatory indexes and endothelial function indexes, and inflammatory response is one of important mechanisms of endothelial function lesion.

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