Tropical Journal of Pharmaceutical Research February 2019; 18 (2): 365-370
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i2.20

Original Research Article

Effect of co-administration of *Linggui zhugan* decoction and Western medicine on inflammatory cytokines, and immune and cardiac functions of patients with chronic heart failure

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Sent for review: 10 November 2018 *Revised accepted: 21 January 2019*

Abstract

Purpose: *To investigate the combined effect of Linggui zhugan decoction and Western medicine on inflammatory cytokines, and immune and heart functions of patients with chronic heart failure (CHF), and the underlying mechanism(s).*
Methods: *Patients with CHF (n = 140) were randomly assigned to two groups: control group and study group (70 per group). Patients in the control group were treated with furosemide, metoprolol, spironolactone, isosorbide mononitrate, trimetazidine and irbesartan, once, twice or thrice daily. Study group patients were treated with a combination of Linggui zhugan decoction, in addition to the treatment given to the control group. Levels of inflammatory cytokines, T-lymphocyte subsets and cardiac function were determined by enzyme-linked immunosorbent assay (ELISA), flow cytometry and color Doppler echocardiography, as appropriate. Clinical effectiveness for the two groups were also assessed.*
Results: *Following the treatments, the serum levels of interleukin-6 (IL-6), interleukins-18 (IL-18) and tumor necrosis factor α (TNF-α) significantly decreased in the study group, when compared with the control group (p < 0. 05). However, the study group had markedly higher levels of CD3+ T, CD4+ T, and CD4+ T /CD8+ T than the control group (p < 0. 05). In addition, CD8+ T was significantly lower in the study group than in control group (p < 0. 05). Patients in the study group had significantly higher total treatment effectiveness than those in the control group (p < 0. 05).*
Conclusion: *These results suggest that co-administration of decoction of Linggui zhugan and western medicine improves ventricular function and reduces clinical symptoms in patients with CHF via a mechanism involving inhibition of inflammatory reactions.*

Keywords: *Chronic heart failure, Linggui zhugan decoction, Inflammatory cytokines, T- lymphocyte subsets, Cardiac function*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Chronic heart failure (CHF) is a clinical condition caused by changes in cardiac structure and function. Abnormalities in structure and function of the heart are due to lowered cardiac output and/or increased intra-cardiac pressure at rest or stress. Treatment of CHF not only improves

symptoms such as dyspnea, but also prevents the development of myocardial remodeling [1]. Despite advances in the treatment of CHF, approximately 23 million people worldwide have been reported to suffer from the disease, and prognosis remains poor [2].

Pulmonary hypertension is a major determinant of poor prognosis in CHF [3]. Pulmonary vascular occlusion is the most common mechanism of pulmonary hypertension in heart failure, and it is due to an increase in pulmonary venous pressure caused by increased chronic left ventricular filling pressure. Pulmonary hypertension may be completely or partially reversed by reducing blood volume and/or improving left ventricular function, and restoring normal left ventricular filling pressure. The partial reversal is due to reduced nitric oxide (NO) utilization in the mitral valve and an imbalance in the expression of endothelin. The reversibility is uncertain, even after returning to normal left hemodynamics [4]. Pulmonary vasoconstriction and remodeling is another mechanism of pulmonary hypertension, and it is associated with increased pulmonary vascular resistance (PVR). The "Golden Triangle" treatment plan consists of angiotensin receptor antagonists (angiotensin-converting enzyme inhibitors), beta blockers and aldosterone system antagonists. However, the side effects are enormous and it is not clinically effective [5].

In Traditional Chinese Medicine (TCM), CHF is believed to be a symptom of the virtual standard and deficiencies of *yin*, *qi*, and *yang* [6]. *Linggui zhugan* decoction was first reported by Zhang *et al*, and shown to significantly improve heart function and clinical effectiveness in patients with CHF [7]. The etiology of CHF is not yet fully elucidated, but there is increasing evidence that inflammatory response plays a role its development [8]. The current study was aimed at investigating the combined effect of *Linggui zhugan* decoction and Western medicine on inflammatory cytokines, and immune and cardiac functions in patients with CHF, and the underlying mechanism.

METHODS

Patients' profile

Patients with CHF (140 cases) were recruited over a 2-year period for this study and randomly assigned to two groups of 70 patients each: control group and study group. There were 43 men and 27 women aged 30 to 78 years (mean age = 52.42 ± 5.64 years) in the control group. The study group consisted of 41 men and 29 women aged 35 to 75 years (mean age = 54.62 ± 5.32 years). The course of disease in the control group ranged from 3 to 10 years (mean course = 6.21 ± 1.04 years), and 4 to 10 years (mean = 6.15 ± 1.24 years) in the study group. The patients were staged according to NYHA classification. The number of patients in grades II, III and IV were 25, 30 and 15, respectively (for control group); and 26, 28 and 16, respectively, for the study group. There were no significant differences between the profiles of the patients in the two groups.

This study received approval from the Ethical Committee of TCM Zhejiang Province Hospital (approval no. TCMZJ2016-891), and was carried out in line with the guidelines of Helsinki Declaration of 1964 as amended in 1996 [9].

Inclusion and exclusion criteria

The included patients were: (1) patients diagnosed with CHF on the basis of 2014 Guidelines for the Diagnosis and Treatment of Heart Failure in China [10]; (2) patients whose conditions fell within grades II and IV of NYHA classification; (3) patients whose diagnosis based on TCM symptoms complied with the "Guidelines for Clinical Research of New Drugs in TCM: Diagnostic Criteria for Chronic Heart Failure" [11]; and (4) those who signed written informed consent forms. The exclusion criteria were: (1) patients with severe liver and kidney dysfunction; (2) patients who suffered acute myocardial infarction or unstable angina before commencement of study; (3) pregnant or lactating women; (4) patients who were allergic to drugs used in the study; and (5) patients who did not sign written informed consent.

Treatment regimen

Control group

Furosemide (20 mg/kg, two times a day), metoprolol (25 mg/kg, two times a day), spironolactone (20 mg/kg, once daily), isosorbide mononitrate (20 mg/kg, twice daily), trimetazidine (20 mg/kg, thrice daily), irbesartan (150 mg/kg, thrice daily) were orally administered for 2 weeks.

Study group

Decoction of *Linggui zhugan* was given, plus the treatment in the control group. The decoction consisted of 30 g of medlar, 20 g of *Atractylodes* rhizome, 15 g of medlar, 10 g of *Guizhi,* 3 g of *Zhigancao, Salvia miltiorrhiza* (30 g), *angelica* (10 g), safflower (10 g), *yang* deficiency *psoralen*

(12 g), dried ginger (6 g), *Ophiopogon japonicus* (3 g), *yin* deficiency + habitat (15 g), *Ophiopogon* (10 g), and *qi* deficiency syndrome + jaundice (30 g). This decoction was administered at a dose of 200 ml/day (100 ml morning and 100 mL in the evening) for 2 weeks.

Evaluation of serum inflammatory factors

Prior to treatment and 2 weeks post-treatment, 4 mL of fasting venous blood was taken for assay of serum levels of IL-6, IL-18 and TNF-α using ELISA kits as per the manufacturer's instructions.

Assessment of immune function

Flow cytometry was used for determination of $CD3^+$ T, $CD4^+$ T, and $CD8^+$ T in T- lymphocyte subsets.

Determination of cardiac function

Left ventricular end-diastolic diameter (LVED) and left ventricular ejection fraction (LVEF) were measured using color Doppler echocardiography before and after treatment.

Determination of clinical effectiveness

The clinical effectiveness was classified into three: *remarkably effective, effective* and *ineffective*. The conditions applicable to each classification were: *remarkably effective*: clinical symptoms improved significantly after treatment, and the scores decreased by ≥ 70 %; *effective*: clinical symptoms improved after treatment, and the scores decreased by ≥ 30 but < 70 %; *ineffective*: clinical symptoms after treatment were not improved or were even aggravated, and the scores were reduced to < 30 %. Clinical effectiveness based on the NYHA classification are: *effective*: heart function improvement ≥ 1; *ineffective*: heart function improvement < 1; and *deterioration*: heart function deterioration ≥ 1. Total effectiveness (TE) was calculated as shown in Eq 1.

$$TE\ (\%) = (RE + E)100 \ldots\ldots\ldots (1)$$

where TE is effectiveness, RE is *remarkably effective,* and E is *effective*.

Statistical analysis

Numeric data are presented as mean ± SEM. Group comparison was done with Student *t*-test and chi-squared test. All statistical analyses were carried out with SPSS version 21.0. Statistical significance was fixed at $p < 0.05$.

RESULTS

Effect of treatment on serum inflammatory factors

Table 1 shows that before treatment, serum IL-6, IL-18 and TNF-α levels were comparable between both groups ($p > 0.05$). However, after 2 weeks, the serum levels of these inflammation factors were significantly decreased, and were markedly lower in the study group patients than in controls ($p < 0.05$).

Table 1: Pre- and post-treatment levels of inflammatory cytokines (mean ± SD, n = 70)

Group	Time point	IL-6 (ng/L)	IL-18 (pg/mL)	TNF-α (ng/L)
Control	Pre-treatment	24.08 ± 3.91	97.47 ± 10.32	274.33 ± 32. 91
	Post-treatment	16.77 ± 3.06[a]	55.63 ± 7.03[a]	198.62 ± 29.47[a]
Study	Pre-treatment	24.43 ± 4.18	97.82 ± 9.64	275. 59 ± 33. 64
	Post-treatment	12.48 ± 2.52[ab]	45.25 ± 6.73[ab]	162.92 ± 24.13[ab]

[a]$P < 0.05$, relative to value before treatment; [b]$p < 0.05$, relative to control

Effect of treatment on T- lymphocyte subsets

The results on Table 2 reveal that pre-treatment values of CD3+ T in both groups were comparable ($p > 0.05$), but were markedly decreased post-treatment, while CD3+ T, CD4+ T, and CD4+ T /CD8+ T in the study group were markedly upregulated, relative to control values. However, CD8+ T was markedly downregulated in the study group patients, when compared to control patients ($p < 0.05$).

Effect of treatment on cardiac function

As shown in Table 3, prior to treatment, LVED and LVEF were similar in the study and control patients ($p > 0.05$). However, post-treatment LVED was decreased significantly, while LVEF was significantly enhanced in both groups ($p < 0.05$). Comparison of post-treatment LVED and LVEF between both groups revealed significantly lower values of LVED and markedly higher LVEF values in the study group of patients, relative to control patients ($p < 0.05$).

Clinical effectiveness

The total effectiveness was markedly higher in study group patients than in control patients ($p < 0.05$; Table 4).

Table 2: Levels of T- lymphocyte subsets in patients (n, %)

Group	n	Time point	CD3+T	CD8+T	CD4+T /CD8+T
Control	70	Before treatment	49.08 ± 6.13	28. 41±4.18	0. 80±0. 35
		After treatment	52.83±6.06[a]	28.33±3.87[a]	1.37±0. 42[a]
Study	70	Before treatment	49.11±5.94	28. 67±4.61	0.84±0. 36
		After treatment	65.84±6.14[ab]	23.64±4.11[ab]	1.99±0.51[ab]

[a]$P < 0.05$, when compared with the value before treatment; [b]$p < 0.05$ when compared with control group

Table 3: Cardiac functions of patients before and after treatment (mean ± SD, n = 70)

Group	LVED (mm)		LVEF (%)	
	Before treatment	After treatment	Before treatment	After treatment
Control	62.14±6.03	54.66±5.03[a]	40.31±4.14	47.27±4.99[a]
Study	62. 28±6.16	43.88±4.26[ab]	41.22±3.95	53.96±5.56[ab]

[a]$P < 0.05$, relative to value pre-treatment; [b]$p < 0.05$, relative to control

Table 4: Clinical effectiveness in both groups (n, %)

Group	n	Remarkably effective	Effective	Total ineffective	Total Effectiveness
TCM symptoms					
Control	70	16 (22.86 %)	35 (50.0 %)	19 (27.14 %)	72.9 %
Study	70	22 (31.43 %)	42 (60.0 %)	6 (8.57 %)	91.4 %
X^2		-	-	-	8.23
p		-	-	-	0.004
Cardiac function					
Control	70	18 (25.71 %)	31 (44.3 %)	21 (30.00 %)	70.00 %
Study	70	20 (28.57 %)	39 (55.7 %)	11 (15.71 %)	84.29 %
X^2					4.05
p					0.044

DISCUSSION

In China, the incidence of CHF is on the rise due to aging population and changing lifestyles. Studies have shown that the age of onset of CHF in China is lower than those of Europe and USA, and the period of hospitalization is relatively longer, thereby adversely affecting the quality of life of sufferers. The "Golden Triangle" treatment is the major strategy for CHF. However, some drugs that are often used such as diuretics may induce electrolyte imbalance, while angiotensin causes hyperkalemia, thus limiting their widespread use in clinical practice [12]. In recent times, the integration of Chinese and Western medicine for the treatment of CHF has become quite popular due to advances in research on CHF. According to TCM, CHF belongs to a class of diseases described as "heart palpitations", "heart water", "sipping", "edema", and "hepatitis syndrome".

Although modern medicine recognizes different clinical causes and pathogenesis of CHF, evidence of the virtual standard is acknowledged by most Chinese medical doctors, and the main cause is *yin*, *qi* and *yang* deficiencies. Decoction of *Linggui zhugan* consists of medlar, cassia twig, *atractylodes*, medlar, zealand and *ginseng licorice,* and it effectively promotes blood circulation [13]. Recent pharmacological studies have demonstrated that *Poria*, *Tinglizi*, Zeeland, and *Salvia* exert potent effects on cardiac function [14]. *Salvia miltiorrhiza* enhances myocardial adenosine triphosphate (myocardial ATP) levels [15]. The pathogenesis of CHF has not been fully elucidated, but it is believed that immune-inflammatory responses play important roles in the progression of the disease. Interleukins-6 (IL-6) and 18 (IL-18) induce the release of a huge amount of nitric oxide in the myocardium, promote apoptosis of cardiomyocytes, and inhibit the contraction of cardiac papillary muscles. Tumor necrosis factor α (TNF-α) exerts negative inotropic effects on the myocardium and papillary muscles, inhibit left ventricular systolic function, while promoting ventricular remodeling [16].

In the present study, the levels of serum IL-6, IL-18 and TNF-α in the study group were significantly lower than those in the control group. However, CD3+ T, CD4+ T, and CD4+ T /CD8+ T levels were significantly higher in the study group than in control group, while CD8+ T and LVED were markedly lower in the study group. In contrast, higher LVEF and higher total effectiveness were seen patients in the study group, when compared with those in control group. These results appear to suggest that

treatment with decoction of *Linggui zhugan* may make up for the limitations of Western medicine, thereby improving heart function. This is in agreement with previously reported findings.

Poria polysaccharide, the main active ingredient in *Linggui zhugan* decoction, promotes the expressions of IL-1β, IL-6 and TNF-α in rats with acute pancreatitis [17]. It also enhances the proportion of Type 1 T helper cells-to-Type 2 T helper cells (Th1/Th2) in peripheral blood of immune-suppressed mice, thus improving immune function [18]. Extract of *Guizhi* reduces the negative expression of IL-6 in abdominal aorta of rats with atherosclerosis [19]. It has been reported that decoction of *Linggui zhugan* downregulated TNF-α and serum level of IL-1β in the heart of rats with CHF in a dose-dependent manner [20].

Limitations of the study

The likely limitations of this study include: (1) lack of in-depth analysis of the possible mechanism of action of *Linggui zhugan* decoction and the correlation between cardiac function, inflammatory factors and immune function; (2) the use of small sample size and a single race/population.

CONCLUSION

These results demonstrate that the decoction of *Linggui zhugan* improves ventricular function while minimizing the clinical symptoms of patients with CHF, by inhibiting inflammatory reactions.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

This work was done by the authors named in this article and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Ji Yunxi; He Xin and Ji Yunxi collected and analyzed the data, while He Xin wrote the manuscript. All authors read and approved the manuscript prior to publication.

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