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Impact of poria, cinnamon twig, bighead *Atractylodes* and licorice decoction with pepperweed and jujube lung-draining decoction on ANP, BNP and ANG II in rats with chronic heart failure: an investigative study.

Kai Li, Qi Zhang*, JianHu Xu, Ziqing Yang, Xiaotong Ren, Leibing Li, Chiqiong Luo, Ye Xiao, Lu Xu, Linxian Li

Chengdu University of Traditional Chinese Medicine, Chengdu Shi, Sichuan Sheng, PR China

Abstract

Objective: This study aims to investigate the impact of Poria, Cinnamon Twig, Bighead *Atractylodes* and Licorice Decoction (PCBLD) with Pepperweed and Jujube Lung-Draining Decoction (PJLD) on Atrial Natriuretic Peptide (ANP), Brain Natriuretic Peptide (BNP) and Angiotensin (Ang) II in Chronic Heart Failure (CHF) rats, to further shed light on the effective mechanism of classic Chinese herbal formulas for CHF.

Materials and methods: Ninety Wistar rats were randomly allocated into modeling group (Group A) and blank control group (Group K). Animals in group A were prepared with Adriamycin (Adr) injection into their abdomen for CHF model. Six weeks after modeling, the CHF rats were randomly aligned to blank modeling group (Group M), Warming Yang and Desolving Phlegm group (Group W), Resolving Phlegm by decoction removing Guizhi group (Group Q). Rats in group W and Q were intragastrically administrated corresponding decoctions at 2 h after the final Adr injection, but not for group M or K. The herbal medicines were given once a day for 4 consecutive weeks. The levels of ANP, BNP and Ang II were tested after 4 weeks treatment.

Results: The level of ANP, BNP and Ang II in group M was significantly elevated, compared with group K ($P<0.01$), after treatment, the levels of BNP and Ang II in group W and Q were significantly reduced, respectively compared with group M ($P<0.01$). But after treatment, there was no significant change for the levels of ANP in group W or Q as compared with group M ($P>0.05$).

Conclusion: PCBLD with PJLD can improve the level of BNP and Ang II of CHF rats, which could be a potential mechanism of the classic formula treating CHF.

Keywords: Chronic heart failure (CHF), Poria, Cinnamon twig, Bighead *Atractylodes* and licorice decoction, Pepperweed and jujube lung-draining decoction, Atrial natriuretic peptide (ANP), Brain natriuretic peptide (BNP), Angiotensin (ANG) II.

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Introduction

Disorder of internal fluid circulation, typically explained as water retention by etiological theory of traditional Chinese medicine, is marked by the pathological product deposited in some certain parts of the body. When it forms, it could interfere with the normal function of organs and meridians, leading to further organic dysfunction. By that, "the secondary pathogeny" refers to the water retention. According to the location of water retention, the disorder varied with different location inside the body, which was literally named as thoracic rheum (Zhi Yin), pleural rheum (Xuan Yin), subcutaneous rheum (Yi Yin), phlegm rheum (Tan Yin) in synopsis of Golden Chamber (Jin Gui Yao Lue). Since the pathogenic water retention is always troublesome and complicated, manifesting as a mess of obstacle diseases with the respiratory, the digestive, the cardiovascular, the urinary system including chronic congestive heart failure, pleural effusion, bronchial asthma, uremia and so forth. While plenty of common and intractable diseases in modern medicine, such as chronic congestive heart failure, pleural effusion, bronchial asthma and uremia are well correlated with water retention, a strong focus on dissolving phlegm and promoting water metabolism is in an urgent need as regarded to the corresponding treatment.

Considering the physiologic and pathogenic characteristic of water retention, Zhang Zhongjing, who was reputed as medical sage in ancient China, put forward his unique therapeutic strategy that the diseases induced by water retention should be administrated by warm herbs, which has been taken as the standard treatment for water retention by the TCM practitioners in later generations. Poria, Cinnamon Twig, Bighead *Atractylodes* and Licorice Decoction, is applied as the representative formula for his therapeutic strategy. With the

function of warming yang, invigorating spleen and eliminating water retention, it focuses on warming yang so as to strengthen the vital energy of the body. And another classical formula Pepperweed and Jujube Lung-Draining Decoction functions on discharging water retention in the lung and highlights on eliminating the symptomatic manifestations. The compatible employment of these two formulas has the function of warming yang and dissolving water retention. The combination is often used to treat many diseases caused by water retention located in different organs, such as chest and diaphragm, heart and lung, stomach and spleen, and so on, and seems efficacious through centuries. The investigation of the mechanism of the above two formulas could help not only to relieve clinical symptoms, but also effectively prevent the recurrence of water retention, thus it is of importance to study the mechanism of the two formulas for water retention. The significance of the research is for both clinical application and academics.

Materials and Methods

Materials

Experimental animals: Ninety healthy adult Wistar rats (male is 45 and the female 45, 8-10 weeks old, 220~250 g) with SPF cleaning grade were all provided by Chengdu Dashuo Biotechnology Co., Ltd. (Chengdu, China, certificate N.SCXK (Chuan) 2008-24). The temperature was normal. All the animals drunk water freely, and were fed by standard synthesis food for rats. This research was approved by the Animal Ethical Committee of Chengdu University of Traditional Chinese Medicine according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985), the approval number is 2015001.

Raw herbs and main reagents: All the raw herbs elucidated in PCBLD and PJLD were selected and purchased from Chengdu herbal market and then the quality of these herbs were in reliance upon the authentication supported by the Department of Chinese Herb Identification of CDUTCM. Adriamycin for injection (Shenzhen Wan Le Pharmaceutical Co., Ltd. No. 1401E1), Sodium pentobarbital (American Sigma corporation, CAS: 69020100), ELISA kits for ANP, BNP, Ang Ⅱ were manufactured by Abcam corporation and imported by Beijing Yonghui Biotechnology Co., Ltd.

Methods

Grouping: Through the computer random grouping method, 90 Wistar rats were randomly were allocated into group A (80) and group K (control group, n=10). After the procedure of modeling, animals in group A would be further allocated into group M, group W and group Q randomly. The survived rats should be at least 10 when the experiment finished.

Modeling: Chronic congestive heart failure models were induced by Adr used for peritoneal injection [1]. Saline was used to dilute the Adr into 2 mg/ml. The prepared Adr was administered by intraperitoneal injection in accordance with 2 mg/kg. The whole injection process consisted of twice a week at beginning 2 weeks and once a week at later four weeks, namely, there are 8 times for injection and 16 mg/mg in total.

Preparation of the decoction and administration

PCBLD is composed of Fuling (Poria), Guizhi (Ramulus Cinnamomi), Baizhu (Rhizoma Atractylodis Macrocephalae), and Gancao (Radix Glycyrrhizae) in proportions of 4332:1 and PJLD include Tinglizi and Dazao in ratio of 43 [2]. Soaking all the raw herbs for 60 min and then decoct them twice. For the first cooking 2640 ml water was used and 1980 ml for the second cooking. The time length for decoction was strictly controlled. For the first cooking, slight boiling should be maintained by temperature controlled electric furnace for 60 min after boiling, while 30 min for the second time. Then the decoctions were merged and filtrated with degreasing cotton. The vacuum concentration technique was adopted to make the merged and filtrated decoction into 200% concentration and make sure that there are 2 g raw herbs in each millilitre. 0.5 g sodium benzoate was added into the prepared decoction which is accordance with Pharmacopoeia of the People's Republic of China (2012 version) stipulated that 1000 ml liquid should contain 3 g sodium benzoate. Finally the decoction was stored in the clear bottle and at temperature of 4°C. The same preparing process for LGZGD without Guizhi.

Intragastric administration should be implemented 2 h after the last injection of Adr and then once a day for 4 consecutive weeks in the amount of 12g/kg.d (LGZGD and TDXD) for Group M and 10g/kg.d (LGZGD and TDXD without Gui Zhi) Group Q. For Group M and Group K, there is no any treatment.

Sample preparation

After treatment for 4 weeks, the rats were anesthetized and weighed. 2 ml blood samples from the carotid artery were collected and were centrifuged in the speed of 4000 r/min for 10 min. The supernatants were collected and stored in the temperature of -20°C.

Testing method: The plasma level of ANP, BNP, Ang II were tested by ELISA. The concrete steps are as follows: (1) Adding sample: Blank holes (blank control holes, no sample and enzyme labeling reagent, and the other steps are the same) are set respectively, and the sample hole shall be measured. In the enzyme labeled plate, the sample hole was added with a sample dilution of 40 μL, and then the sample to be tested was 10 μL (the final dilution of the sample was 5 times). Add the sample to the bottom of the microplate hole. Shake it gently and mix it evenly; (2) Warming up: Seal plate with film and incubation for 30 min at 37°C; (3) Wash: remove the sealing board and discard the liquid, then dry it. Filling it with detergent, leave it in 30 s. Repeat it 5 times; (4) Adding enzymes: adding 50 L per enzyme label to each pore, except the blank hole. Then repeat the warming up and wash; (5) Color rendering: each hole add color agent A50 μL, and then add color rendering agent B50 μL, avoiding light for 15 min at 37°C; (6) Termination: termination of the reaction by 50 μL

per pore; (7) Measurement: absorbance was measured by wavelength of 450 nm (OD).

Statistical methods

Statistical analyses were conducted with SPSS18.0. Descriptive statistics were expressed as mean ± SD for continuous variables. Differences in laboratory assay data were compared with the analysis of one-way ANOVA. And pairwise comparisons using LSD test. Two-sided p value of 0.05 was considered statistically significant.

Results

General condition and mortality rate

Throughout the experiment, there was no death in the blank control group. As to Group A with 80 rats, in the first 3 weeks of modeling, most of the rats put on body weight, from the beginning of the fourth week, the body weight is generally reduced with the phenomenon of drug poisoning which contains poor mental state, increased eyelid secretions, less activity, less eating, soft hair, hair loss, faster breathing, preferring to gather and even diarrhoea appeared in some rats. Hair removal in female rats was more serious than male. The hair loss is particularly serious in 13 female rats in which there are large areas like neck, shoulder and root of tail.

At the end of the modeling, 8 rats died accounting for the 10% death rate. From the treatment to the end of the experiment, there are 6 rats died in group M with 26.09% death rate, 7 rats died in group Q with 30.43% death rate and 8 rats died in group W with 33.33% death rate. The mortality rate of the treatment groups is higher than that of the model group. We believe that this is related to the side effects of Adriamycin and the way of intragastric administration. One of the adverse reactions of the drug is esophageal inflammation which was aggravated by intragastric administration, stimulation to esophagus. This phenomenon in the late period of the experiment is more obvious.

Impacts on plasma ANP of rats with CHF

Compared with group K, the ANP level of group M was significantly increased (P<0.01), while there are no obvious difference between group M, W and Q (P>0.05) (Table 1).

Table 1. *Impacts on plasma ANP of rats with CHF ($\bar{x}$ ± s).*

Group	n	ANP (ng/L)
Group K	10	103.83 ± 26.62
Group M	10	135.10 ± 16.65$^{\Delta\Delta}$
Group Q	10	124.20 ± 20.44$^{\Delta}$
Group W	10	133.19 ± 13.92$^{\Delta\Delta}$
F		5.13
P		0.0047

Compared with group K, $^{\Delta}$P<0.05; $^{\Delta\Delta}$P<0.01.

This research indicates that compared with group K, the ANP level of group M was significantly increased (P<0.01), which demonstrates endocrine abnormality of hearts in group M rats and symbolizes the success of modeling. Compared with group K, the ANP level of group W and group Q was significantly increased (P<0.05 or P<0.01), which illustrates that PCBLD and PJLD and PCBLD and PJLD without Guizhi has little impact on ANP of CHF rats.

Impacts on plasma BNP of rats with CHF

Compared with group K, the BNP level of group M was significantly increased (P<0.01), while compared with group M, the BNP level decreased obviously in group W and group Q (P<0.01) (Table 2).

Table 2. *Impacts on plasma BNP of rats with CHF ($\bar{x}$ ± s).*

Group	n	BNP (ng/L)
Group K	10	126.83 ± 28.25
Group M	10	156.4 ± 9.42$^{\Delta\Delta}$
Group Q	10	127.85 ± 21.83**
Group W	10	131.22 ± 18.19**
F		4.63
P		0.0077

Compared with group K, $^{\Delta\Delta}$P0.01; Compared with group M, **P<0.01.

This research reveals that compared with group K, the BNP level of group M was significantly increased (P<0.01), which symbolize the success of modeling of CHF and the group M rats are in the transitional period of the heart failure from the compensatory stage to the decompensated one.

Compared with group M, the BNP level of group W and group Q was significantly decreased (P<0.01). The result illustrates that, no matter Guizhi was added into the decoction or not, they could decrease the BNP level, and lowering the BNP level implied one contributive mechanism of improving the heart function of rats with CHF.

Impacts on plasma Ang of rats with CHF

Compared with group K, the Ang II level of group M was significantly increased (P<0.05), while compared with group M, the Ang II level decreased obviously in group W and group Q (P<0.05) (Table 3).

Table 3. *Impacts on plasma Ang of rats with CHF ($\bar{x}$ ± s).*

Group	n	Ang II (ng/L)
Group K	10	2.31 ± 0.64
Group M	10	2.82 ± 0.42$^{\Delta}$

Group Q	10	2.33 ± 0.44*
Group W	10	2.25 ± 0.30*
F		3.2
P		0.0346

Compared with group K, ΔP<0.05; Compared with group M, *P<0.05.

The research reveals that compared with group K, the Ang II level of group M was significantly increased (P<0.05). Compared with group M, the Ang II level of group W and group Q was significantly decreased (P<0.05). The result illustrates that both decoctions have antagonistic action to the activated RAAS system induced by CHF, through which sodium and water retention can be relieved and cardiac function improved.

Discussion

CHF is the severe stage of various heart diseases which could present various complicated symptoms. According to TCM, the understanding of the basic pathogenesis of CHF has been reliable. It definitely holds that the disorder features as qi and yang deficiency, blood stasis, water retention, qi and yin deficiency, which consists a circular process. Deficiency of heart qi and yang is the pathological basis of the disease. Blood stasis and water retention are the results and products of the disease which are also the main factors for induction and aggravation [3]. Recorded in synopsis of golden chamber performing the action of eliminating phlegm and water retention, PCBLD and PJLD is efficacious in warming yang, strengthening spleen, dissolving phlegm and water retention, thus they are usually applied for CHF in clinics [4-9]. To identify the functions of the essential component herb strongly warming yang in the treatment, the authors respectively designed PCBLD and PJLD group (group W) and PCBLD and PJLD with Guizhi subtracted group (group Q) and eventually the following outcomes were investigated .

The impact on plasma ANP

ANP is a kind of polypeptide hormone released by atrial muscle cells, which mainly acts on vascular smooth muscle and myocardium, and has a significant expanding action of blood vessels, lowering blood pressure, inhibiting the Renin Angiotensin Aldosterone System (RAAS), generating sodium loss and diuresis [10]. It is indicated that the concentration of ANP in blood of patients with CHF is 5-10 times of normal people [11]. The occurrence and severity of ANP were highly correlated with CHF. The secretion of ANP increased with the deterioration of heart condition, therefore, it might be regarded as an index to judge the severity of CHF and to detect the effect of medicine for CHF [12,13].

The impact on plasma BNP

BNP, akin to the ANP, is another member of the natriuretic peptide system, which is synthesized by the ventricular muscle cells and defined as a kind of cardiac neurohormone. Due to its limited expression in the ventricle, it can reflect the status of ventricular disease more rigorously. When patients suffer from heart failure, their cardiac systolic and diastolic function would decline, accompanied by a lot of secretion of BNP. It has been confirmed that BNP is the most significant marker of left ventricular systolic dysfunction [14]. When CHF occurs, the level of serum BNP would significantly increase, and the level of BNP is positively correlated with the severity of CHF [15,16]. BNP is the most sensitive indicator to estimate severity of CHF, disease prognosis and drug efficacy [17]. In addition, BNP is mainly secreted in the later decompensated stage, which could be considered as an symbol of the transitional period of the heart failure from the compensatory stage to the decompensated one [14].

The impact on plasma Ang II

Ang II, one of the most important active hormone in RAAS system, is a powerful vasoconstrictor, which could stabilize the blood pressure and blood supply to the vital organs, increase returned blood volume and cardiac output. It has been widely recognized that Ang II would increase in CHF cases, but the persistence and excessive increased Ang II could exacerbate CHF [18]. The level of Ang II could reflect the severity of the CHF after decompensated stage [19].

Conclusion

PCBLD and PJLD and PCBLD and PJLD without Gui Zhi could significantly improve the level of BNP and Ang in serum of CHF rats, and improve cardiac neuroendocrine function in CHF rats.

Under the condition of this experiment, there is no significant difference between two treatment groups for the rats. It is speculated that this may be related to the factor that syndrome differentiation was not induced into the model. The model of the pleural effusion is consistent with the characteristics of pleural rheum, which refers to the water suspended in the chest, but not in line with the “Yang deficiency syndrome’ model at the same time. It reminds that if the researchers want to observe the difference of curative effect of classical formulas between original ones and modified ones, we should establish the model of TCM syndromes for the animals in the experiment.

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*Correspondence to

Qi Zhang

Chengdu University of Traditional Chinese Medicine

Chengdu Shi

Sichuan Sheng

PR China