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Original Research

Evaluation of Danzhi Xiaoyao powder and amlodipine sustained-release tablets in follow-up treatment of the hypertensive crisis and the interleukin-6 gene expression

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Abstract: Danzhi Xiaoyao powder (DXP) is an herbal formula with eight different herbs. This herbal medicine can play multiple roles in various disease treatments through its several components. In this study, the effect of DXP was evaluated on the treatment of hypertensive patients with and without amlodipine. For this purpose, 252 patients were studied with high blood pressure. The 126 patients received DXP, and the others received DXP + amlodipine for four weeks. Besides demographic and biochemical assessments (gender, BMI, age, SBP, DBP, etc.), the expression of the interleukin-6 gene was evaluated in these two groups. The results showed that the blood pressure decreased by DPX, and there was no significant difference in control of blood pressure by DPX and DXP + amlodipine. But it did not affect interleukin-6 gene expression. Therefore, it can be concluded that this drug probably uses a different mechanism than amlodipine to control blood pressure.

Key words: Amlodipine; Danzhi Xiaoyao Powder; Hypertensive crisis; Interleukin-6.

Introduction

Arterial blood pressure is an important indicator in assessing the cardiovascular status of individuals (1, 2). Blood pressure is affected by two factors: cardiac output and peripheral vascular resistance. Cardiac output is largely controlled by factors such as heart rate and inotropic status that are affected by calcium concentration (1). Environmental resistance is mainly affected by the rate of vascular base contraction, which is highly dependent on the intracellular calcium concentration of vascular smooth muscle. Regardless of the cause, studies have shown that in many patients with hypertension, peripheral vascular resistance increases (3). Important reasons have been suggested that the cause of increased environmental resistance is vascular stenosis due to increased intracellular calcium concentrations. This may be due to an inherited defect in the cell membrane or a genetic change in the structure of the sodium-potassium pump, although hormonal and neurological factors may also play a role in increasing intracellular calcium concentrations and increasing peripheral vascular resistance (4).

According to the above points, it is clear that changes in the concentration of intracellular calcium ions through the effect on environmental resistance and contractile strength of the heart can have significant effects on arterial blood pressure (5). Calcium channel blockers are an important group of drugs that lower blood pressure by reducing peripheral vascular resistance and reducing cardiac activity. The antihypertensive effects of these drugs are more pronounced in patients with hypertension (6). There are currently three classes of calcium channel blockers: Phenylalkylamines, benzothiazepines, and dihydropyridines (7).

Amlodipine is a kind of dihydropyridine that has a strong affinity for smooth muscle calcium channels and acts as dilators of arterial vessels and has little effect on the heart muscle (6). One of the ways that the function of amlodipine can be studied is the effect of this drug on the expression of some related genes. In this regard, many studies have shown that amlodipine increased the expression of the interleukin-6 (IL-6) gene (8, 9). However, the exact molecular mechanisms of the amlodipine on increasing IL-6 gene expression are still unknown (10) but in general, because amlodipine causes inflammation by blocking calcium channels, it increases the expression of the interleukin-6 gene (11). The product of the interleukin-6 gene is one of the most important cytokines. Interleukin-6 is secreted by T cells and macrophages to stimulate the immune response to trauma and tissue damage, and this cytokine, which is secreted by vascular smooth muscle, is directly linked to inflammatory cardiovascular disease (12, 13).

Recently, in addition to chemical drugs, herbal medicines have been considered for the treatment of many diseases, including hypertension. One of the most important of these drugs is Danzhi Xiaoyao powder (DXP). DXP is an herbal formula that consists of eight different herbs: Angelicae Sinensis, Atractylodes macrocephala, Gardeniae Fructus, Koidz. cortex moutan, Licorice., Paeoniae radix alba, Poria cocos wolf., and Radix bupleuri (14). Many researches showed that DXP has a certain effect on different diseases such as breast cancer, neuro-immuno-endocrine system, depres-

sion, hypertensive crisis, etc. (14-17), Therefore, DXP, with or without other drugs (chemical or herbal medicine), has the potential to be a drug for treating many diseases (18). In this study, the effect of DXP in the treatment of hypertension was evaluated in comparison with the combination of this powder and amlodipine on different patients. The effect of this medicine on the expression of the interleukin gene was also investigated.

Materials and Methods

Demographic and biochemical assessments

This study was double-blind, controlled with a combination of DXP and amlodipine, and it was performed on 252 hypertension patients. Inclusion criteria were hypertension the diagnosis of it in these patients was based on the criteria of the World Health Organization (19). Informed consent was obtained from all participants before entering the study. Patients were randomly divided into two groups. One group received DXP and the other received combination of DXP and amlodipine for four weeks. For each participant in the study, a questionnaire related to demographic and biochemical assessments was completed before and after the intervention. The variables included gender, BMI (Kg/m^2), age, systolic blood pressure (SBP), diastolic blood pressure (DBS), heart rate (HR), smoking, and drinking. These assessments were performed in the morning, during fasting and when people were dressed lightly and without shoes.

Extraction of RNA from the blood sample

In this study, Four ml of blood in a tube containing K3EDTA anticoagulant was taken from the participants. Peripheral blood mononuclear cells were immediately isolated by density gradient method using ficoll hypaque 1.077 (Sigma-Aldrich, Germany). After washing 2 times with Phosphate-Buffered Saline-PBS, total RNAs were extracted with Plus-RNX solution (Sinaclone, Iran) and they were stored at -80°C until cDNA formation. DNase enzyme (ThermoFisher, USA) was used to treat whole RNA samples for greater purity and removal of genomic DNA contamination. Then, using the cDNA synthesis kit (ThermoFisher, USA), all RNAs extracted at the same concentration of one microgram per microliter were converted to cDNA.

Real-time PCR

Interleukin-6 gene expression was examined by real-time PCR technique using specific primers and probes as follow:

Probe: (FAM)-AGGAGAAATGCCTGAC-GAAGCTCTCCA-(TAMRA)

Forward primer: 5'-GCTCGCCGGCTTCGA-3'

Reverse primer: 5'-GGTAGGTCTGAAAGGCGAA-CAG-3'

β-actin gene was used as a housekeeping or internal control gene. Each real-time PCR reaction consists of: 10µl of Master-mix of TAMRA PCR, 3µl of sample cDNA, 0.8µl of each primer (with 10 pM concentration: Sense and Antisense) and 0.8µl of specific gene probe (with a concentration of 10 pM) and 6µl of nuclease-free water, the total final volume is 20µl. All reactions were performed in duplicate using a Rotor-gene 3000 (Corbett, Australia). The temperature profiles of the reactions included the hold time stage at 95°C for 10 minutes, the degradation stage at 95°C for 15 seconds, the annealing and extension stages at 60°C for 60 seconds for 40 cycles.

Statistical Analysis

Kolmogorov-Smirnov statistical test was used to examine the normal distribution. According to the results of this test, the non-parametric Mann-Whitney U test between the two groups was used for statistical analysis. The relative expression method with formula $2^{-\Delta\Delta CT}$ was used to calculate the results of gene expression. For this purpose, the beta-actin reference gene was used to normalize the expression of the studied genes. Also, using six dilutions prepared from PCR reaction product (from dilution 10^{-4} to 10^{-9}) and drawing a standard curve, the PCR reaction efficiency was measured for each gene and due to the same amount of reaction efficiency (R^2= 0.997) in the case genes, Livak formula in Formula $2^{-\Delta\Delta CT}$ was used to calculate the results. Finally, using SPSS software version 20 was used for statistical analysis and Pad Graph 6 Prism software was used to draw the graphs. A P-value less than 0.05 was considered as a significant result.

Results

General characteristics

252 enrolled patients were separated into 126 cases (those who received DXP) and 126 controls (those who received DXP+amlodipine). The demographic and biochemical assessments of the study population are summarized in Table 1. Men had a higher incidence of

Table1. The demographic and biochemical assessments of 252 patients.

Variable	DXP (n=126)	DXP+amlodipine (n=126)	*P*-value
Gender			
Male (n)	80 (63.49%)	75 (59.52%)	0.682
Female (n)	46 (36.51%)	51 (40.48%)	0.682
BMI (kg/m^2)	26.12 ± 2.37	26.43 ± 3.12	0.701
Age (year)	62.09 ± 11.35	65.29 ± 12.89	0.065
Systolic Blood Pressure (mmHg)	142.08 ± 17.29	139.17 ± 15.64	0.231
Diastolic Blood Pressure (mmHg)	82.14 ± 11.01	80.17 ± 9.31	0.725
Heart Rate (beats/min)	73.64 ± 9.27	72.98 ± 9.66	0.512
Smoking (n)	12 (9.52%)	15 (11.90)	0.398
Drinking (n)	35 (27.77)	51 (40.47)	0.120

hypertension than women which is in agreement with previous studies (20-22). There was no significant difference between the two groups regarding all variables that confirms the two points. The first point is that population selection and randomization are done very well so that the effects of these differences on the main result are minimized. The second point is about the effect of DXP on controlling blood pressure in hypertensive patients, which shows that this drug has been able to control blood pressure.

Interleukin-6 gene expression

Interleukin-6 gene expression and its possible role in DXP function were evaluated. The mRNA expression of this gene in peripheral blood mononuclear cells was evaluated by real-time PCR technique and TAMRA probe. As shown in Figure 1, the expression of the interleukin-6 gene in the DXP and the amlodipine-treated group showed a significant increase compared to the DXP-treated group. Although the expression of the interleukin-6 gene is slightly increased in people who received the DXP, this increase is not significant.

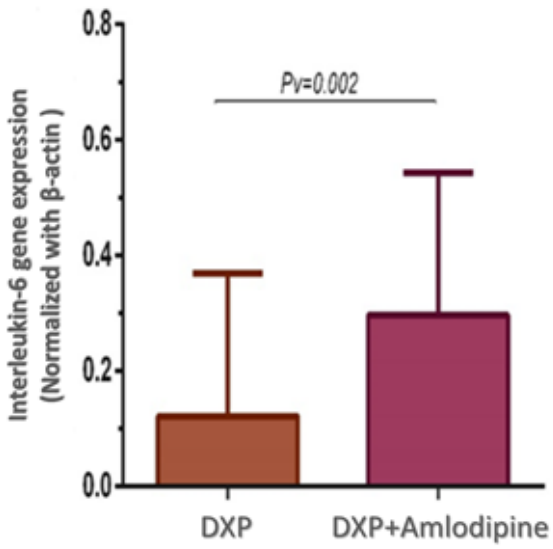

Figure 1. Comparison of interleukin-6 gene expression in the two groups; the group treated with DXP and the group treated with DXP and amlodipine (*Pv*=0.002).

Discussion

During human history, medicinal plants have always had frequent usages in the treatment of illnesses (23-28). Danzhi Xiaoyao powder (DXP) is a Chinese herbal formula that has a certain effect on hypertensive crisis treatment (29). The current results proved the effectiveness of DXP on hypertension treatment. According to the results, DXP was able to reduce systolic blood pressure to an average of 62.09 mm Hg and diastolic blood pressure to about 82.14 mm Hg, which was not a significant difference with a combination of DXP + amlodipine (Table1). Therefore, according to previous studies and the results of this study, this powder can be considered an effective drug in the treatment of patients with hypertension (29-31).

To evaluate the mechanism of DXP action, since it had a similar function to amlodipine in controlling blood pressure, the mechanism of action might have been similar to amlodipine. Therefore, in the present study, the likelihood of the DXP action mechanism was evaluated with amlodipine.

Amlodipine belongs to a group of medicines known as calcium channel blockers; however, it does not interfere with the calcium in the blood. It prevents the walls of blood vessels from constricting by blocking the entry of calcium into the cells, which controls high blood pressure and prevents coronary artery spasm (32). Many researches showed that amlodipine increases the expression of the interleukin-6 gene (33-36).

Interleukin-6 (IL-6) is an important interleukin in the body that is secreted by white blood cells and is involved in inflammatory and immune responses (37). IL-6 is secreted by macrophages, donor L-lymphocytes, B-lymphocytes, and astrocytes, and affecting on plasma cells and lymphocytes (38). The main function of this cytokine is to differentiate plasma cells faster and produce more antibodies (39).

Because amlodipine blocks calcium channels, the body reacts with inflammation and therefore increases the expression of the IL-6 gene (40, 41). To determine whether DXP also increases the inflammatory response by blocking and thus increasing gene expression, we compared the expression of IL-6 in the blood sample to the DXP-treated group and the DXP + amlodipine group. The results showed that DXP, although increased the expression of the interleukin-6 gene, did not have a significant effect on the expression of this gene. Therefore, it is likely that DXP does not use the amlodipine mechanism to decrease blood pressure. Hence, more studies should be done on the mechanism of DXP action on hypertension.

Today, Medicinal herbs have received a lot of attention because of their lower side effects, lower prices, and more availability than chemical drugs (42). One of the most important herbal medicines is Danzhi Xiaoyao powder (DXP) which is involved in the treatment of many diseases, including hypertension (43). The results of this study confirmed the effect of this herbal formula on the treatment of hypertension in comparison to amlodipine. Although in this study we also tried to investigate the action mechanism of DXP through the expression of the interleukin-6 gene, we still need more studies in this area.

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