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당귀작약산이 정상인의 총경동맥 탄력도에 미치는 영향 : 무작위대조군 교차시험

Effects of Dangui-jakyak-san on Common Carotid Artery Elasticity in Healthy Subjects ; A Randomized controlled crossover study

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■ **Objectives** 당귀작약산 단회 복용으로 정상인의 총경동맥 탄력도에 미치는 영향을 평가하고자 하였다.

■ **Methods** 본 연구는 정상인 남성을 대상으로 한 전향적 무작위 대조군 교차시험연구다. 모든 대상자들은 임의로 당귀작약산 복용군과 비복용군인 대조군으로 나누어졌다. 1주일 간격으로 총 2회 방문하며, 첫 번째 방문 시에 당귀작약산 복용군은 당귀작약산 1포(2.5g)과 물을 제공받고, 대조군은 물만 제공받아 복용하였다. 두 번째 방문 시 첫 번째 방문과 반대로 당귀작약산을 복용했던 복용군은 물만 제공받고, 물만 복용했던 피험자들은 당귀작약산 1포와 물을 제공받았다. 물은 100 cc로 매번 동일하게 제공되었다. 모든 대상자들은 복용 직전과 복용 2시간, 4시간 후에 총경동맥 탄력도, 혈압, 총경동맥 내막-중막 두께와 맥박수를 측정하였다.

■ **Results** 총 20명의 정상인 남성이 모집되었으며, 시간에 따라 당귀작약산 복용 후의 총경동맥 탄력도가 대조군에 비하여 유의하게 상승한 것이 확인되었다. 내막-중막 두께, 혈압 및 맥압, 맥박은 유의한 변화가 나타나지 않았다.

■ **Conclusion** 당귀작약산의 단회 복용으로 총경동맥 탄력도가 즉시 개선되는 것을 확인하였고, 이로써 당귀작약산이 동맥 경직도 완화 및 탄성의 개선에 영향을 미친다는 것을 알 수 있었다.

■ **Key words** Dangui-jakyak-san(DJS), Common carotid artery, Atherosclerosis, Arterial stiffness, Korean medicine

I. Introduction

Arterial stiffness is a significant indicator for assessing the structure and function of arterial vessels, because it is considered as initial indication of atherosclerotic cardiovascular disease[1]. Decrease of vascular endothelial function is early stage of increase arterial stiffness[2]. Increased arterial stiffness is one of the early manifestations of adverse degenerative changes within the vessel wall and an important independent predictor of

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cardiovascular disorders[3-5].

Traditional Korean prescription, Danggui-jakyak-san (DJS) from "Synopsis of Prescriptions of the Golden Chamber, GeumGueyoryak" consisted of Paeoniae Radix, Atractylodis lanceae rhizome, Alismatis Rhizoma, Poria (Hoelen), Cnidii Rhizoma, and Angelicae radix. It has been mostly used to eliminate blood stasis syndrome, stimulate blood circulation and relieve pain[6-8].

In the previous studies, DJS improved cognitive impairment, blood microcirculation[9] and stimulated production of progesteron which has neuroprotective effect against certain types of neurodegeneration[10]. Also, there were studies about antioxidant effects of DJS, that lead to antithrombotic properties. This results from increase of releasing platelet nitrous oxide (NO) and inhibiting effects on platelet aggregation and thrombus formation[6, 11].

Although DJS has been reported to have clinical efficacy on cardiovascular diseases[12], there are few studies that assess its function on human, so that still more efforts are needed to evaluate its direct effect on blood circulation. Therefore, this study was designed to find out the effect of DJS on arterial stiffness, using a novel parameter, Corrected circumference strain (CCS).

II. Materials and Methods

2.1. Subjects

A total twenty of healthy male subjects were included in this study. Women were excluded in this study because vascular endothelial function could changed by estrogen level in blood through menstrual phase[13]. The exclusion criteria also included a history of cerebrovascular or cardiovascular disease, diabetes mellitus, hypertension, endocrinologic disease, or psychiatric problems and any diseases during period of study. Every subject was prohibited smoking, intaking caffeine, alcohol, and any medicine which could affect arterial stiffness, within 24 hours before study. Informed consensus was obtained from the study subjects after a full explanation of this study.

2.2. Materials

Danggui-jakyak-san (DJS) is consisted of Paeoniae Radix, Atractylodis lanceae rhizome, Alismatis Rhizoma, Poria (Hoelen), Cnidii Rhizoma, and Angelicae radix. For standardization of material, granules from Tsumura company (Japan) was used.(Table 1.)

2.3. Study design

This study was conducted as a randomized controlled cross-over design. Subjects were allocated into two groups by block randomization with

Table 1. Composition of Danggui-jakyak-san(DJS)

Constitute herbs	Scientific name	Weight(g)
Paeoniae Radix	Paeonia lactiflora	2.0
Atractylodis lanceae rhizome	Atractylodes lancea	2.0
Alismatis Rhizoma	Alisma canaliculatum	2.0
Poria (Hoelen)	Poria cocos Wolf	2.0
Cnidii Rhizoma	Cnidium officinale	1.5
Angelicae radix	Angelica gigas	1.5

excel numbering after screening. All subjects had to visit two times at intervals of 1 week for wash-out period and were equally divided into two groups depending on whether taking DJS or water first. Since randomization and dosing process was conducted by a third person who received research training, this study was designed as investigator-blind study.

At the first visit, baseline values of CCS, carotid intima-media thickness (IMT), systolic blood pressure, diastolic blood pressure and pulse rate were examined after a subject took 5 minutes rest. After examination, one group received a DJS single pack (2.5 g per pack) with water 100 cc, while the other group got only water 100 cc. The measurements were repeated at 2 and 4 hours after DJS or water administration. A week later, at second visit, the two groups were crossovered and took the other item. The same variables were examined as the first visit. Adverse events were monitored during the study. A study protocol is showed on Fig. 1.

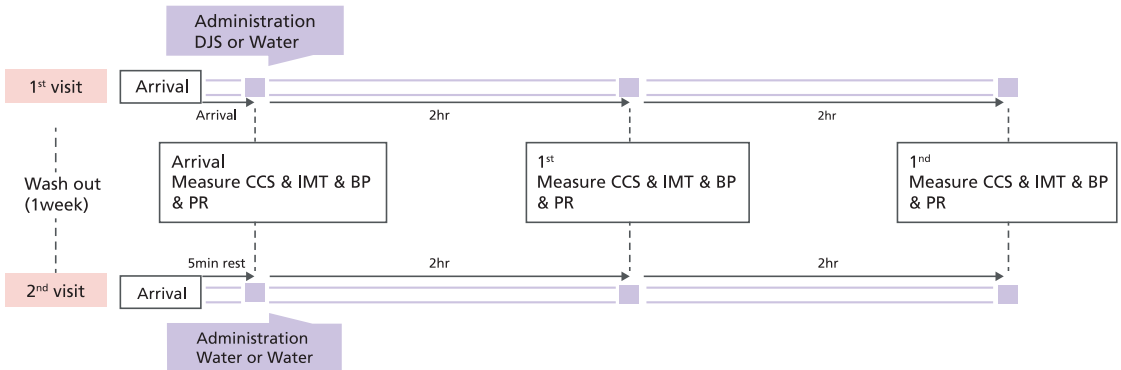

Fig. 1. Study protocol.
DJS, Danggui-jakyak-san; CCS, Corrected circumference strain; IMT; Carotid Intima-media thickness; BP, Blood pressure; PR, Pulse rate

2.4. Measures

At the beginning of the study, systolic and diastolic blood pressures and pulse rates were examined from left brachial artery in sitting position after 5 minutes of rest.

The carotid image was acquired by an Ultrasound Machine, Vivid S5(General Electric Medical System, Milwaukee, WI, USA). This machine is identified as Class IIa by Europe Union-Medical Devices Directive (93/42/EEC), and certified by International Electrotechnical Commission (IEC, IEC60950:2005) and Korean Food and Drug Administration (KFDA, SH11-383).

To measure the common carotid circumference strain values, quantified carotid arterial elasticity, 7.5-10MHz linear transducer was used. During examination, all subjects were required to hold breath for while to minimize the motion artifacts.

The long and short axis views were taken 1cm apart from the carotid bifurcation toward proximal side at both common carotid arteries (CCA). Simultaneously, at least three continuous heartbeats were stored at the average frame rate during examination.

Along the intima-blood interface, a region of interest (ROI) was traced manually on short axis

of the CCA, using a speckle-tracking algorithm. The appropriacy of trace section of the arterial walls were checked by the mechanical system and calculated automatically.

After taking ultrasound image, strain was analyzed with 2D strain software (EchoPac 7.0.0, GE Vingmed Ultrasound). The software spotted the vessel wall and automatically tracked its movement along the cardiac cycle, resulting in strain levels.

The circumference strain was calculated by average of both side strain values, which is also average of 6 regional strain values. As pulse pressure is known as significant variable for circumference strain, CCS has been suggested as a primary outcome: Circumference strain / (Systolic blood pressure; Diastolic blood pressure).

At the same time the carotid intima-media thickness was obtained on the long axis of both sides before the carotid bulb with same transducer and calculated by a virtual caliper built-in software.

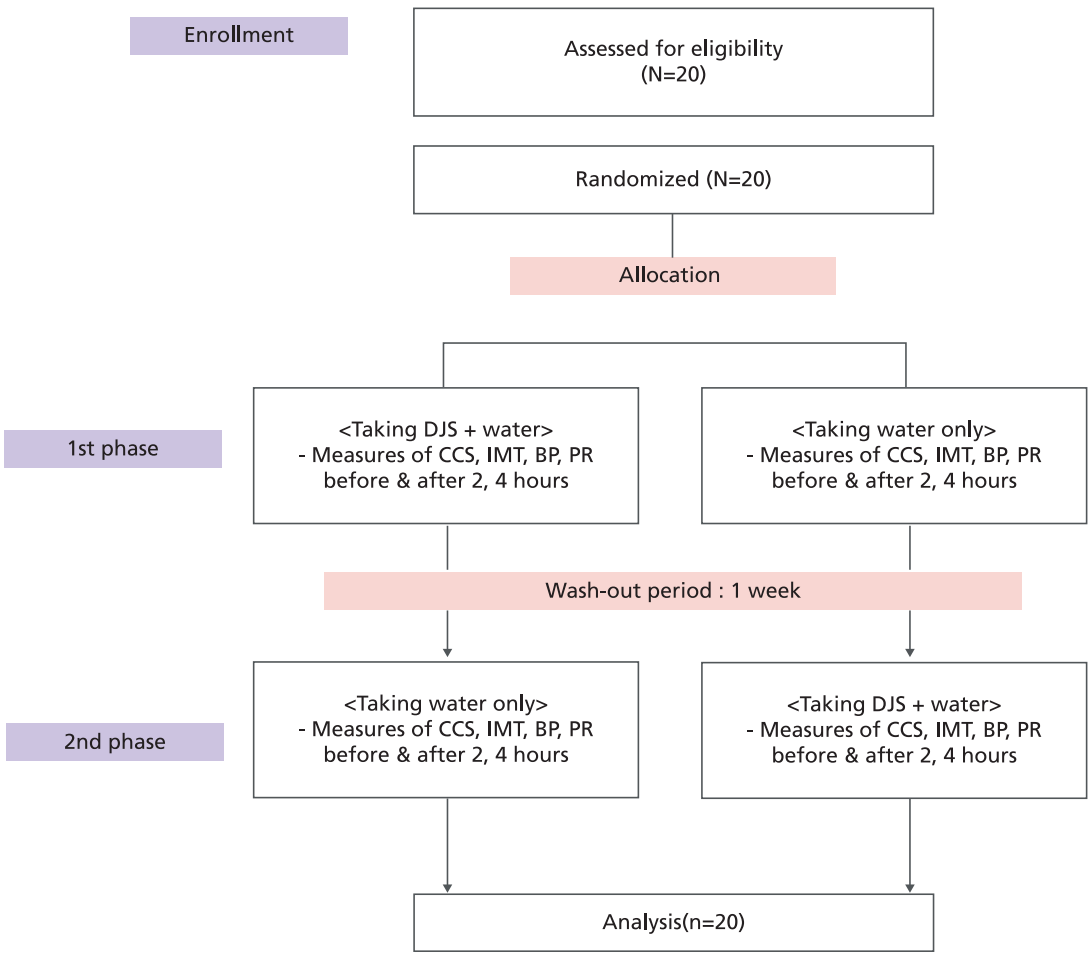

Fig. 2. Flow chart.
DJS, Danggui-jakyak-san; CCS, Corrected circumference strain; IMT; Carotid Intima-media thickness; BP, Blood pressure; PR, Pulse rate

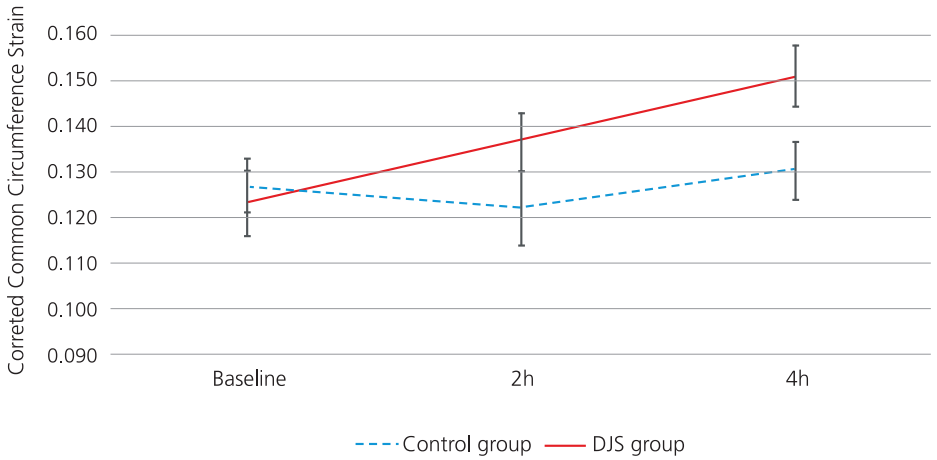

Fig. 3. Changes of CCS over time after Administration
The graph shows the changes of CCS by mean ± standard deviation.
DJS, Danggui-jakyak-san; h, hour; CCS, Corrected Circumference Strain

2.5. Statistical analysis

Every outcome summarized as mean±standard deviation. To compare the difference between the control and the DJS group in baseline, independent t-test was used after the test of normality. To assess continuous variables before and after intervention, repeated-measured ANOVA was used. All of the statistical analyses was performed with the Statistical Package for the Social Sciences version 12.0 for Windows (SPSS, Chicago, Illinois.), and statistical significance was settled if p-value were under 0.05.

III. Results

3.1. Baseline Characteristics

A total of 20 healthy male subjects (mean age, 27.05±1.36 years) were enrolled in this study (Fig. 2) and no one dropped out during the study. There was no significant difference between the two groups in baseline characteristics. (Table 2)

3.2. Carotid arterial Corrected Circumference Strain (CCS)

There was significant increase of CCS in the DJS group (p=0.017, Table 3) while the control group shown no significant changes over time. (Fig. 3.)

Table 2. Baseline characteristic (n=20)

	DJS (n = 20)	Control (n = 20)	p-value*
Corrected Circumference Strain (%/mmHg)	0.125±0.031	0.127±0.026	0.289
Average Intima-Media Thickness (mm)	0.54±0.06	0.51±0.05	0.452
Average Pulse Pressure (mmHg)	64.4±6.7	61.1±8.8	0.215
Average Pulse rate (beats/min)	76.9±12.0	77.3±12.0	0.593

Table 3. CCS measures (%/mmHg) at baseline and 2, 4 hours after administration

	Baseline	After administration		p-value
		2h	4h	
DJS (n=20)	0.123±0.031	0.136±0.029	0.151±0.030	0.017*
Control (n=20)	0.126±0.026	0.122±0.037	0.130±0.030	

DJS, Danggui-jakyak-san.
The data are presented as mean ± standard deviation.
*p-value by independent t-test.

Table 4. Average IMT measures (mm) at baseline and 2, 4 hours after administration

	Baseline	After administration		p-value
		2h	4h	
DJS (n=20)	0.54±0.06	0.52±0.08	0.51±0.06	0.243*
Control (n=20)	0.51±0.05	0.52±0.06	0.51±0.07	

DJS, Danggui-jakyak-san; h, hour; IMT, Intima-Media Thickness.
The data are presented as mean ± standard deviation;
*p-value derived from repeated measure ANOVA.

Table 5. Average pulse pressure (mmHg) measures at baseline and 2, 4 hours after administration

	Baseline	After administration		p-value
		2h	4h	
DJS (n=20)	64.4±6.7	62.6±6.3	58.4±6.0	0.144*
Control (n=20)	61.0±8.8	61.5±5.6	60.1±9.0	

DJS, Danggui-jakyak-san; h, hour.
The data are presented as mean ± standard deviation;
*p-value derived from repeated measure ANOVA.

Table 6. Average pulse rate (beats/min) measures at baseline and 2, 4 hours after administration

	Baseline	After administration		p-value
		2h	4h	
DJS (n=20)	76.9±12.0	76.9±12.1	75.3±11.2	0.854*
Control (n=20)	77.3±12.0	78.5±11.5	75.1±12.3	

DJS, Danggui-jakyak-san; h, hour.
The data are presented as mean ± standard deviation;
*p-value derived from repeated measure ANOVA.

3.3. Changes of secondary outcomes

The average carotid intima-media thickness, pulse pressure defined as the difference between systolic and diastolic blood pressure and pulse rate showed no significant changes over time. (Table 4, 5, 6)

3.4. Adverse effect

All subjects were asked about any adverse reactions such as stomach distension, diarrhea etc. after examination. During entire study period, there was no adverse event in every subjects.

III. Discussion

This study identified that Danggui-jakyak-san (DJS) could increase the arterial elasticity in healthy men, using two-dimensional speckle tracking strain. This strain from two-dimensional speckle tracking is one of the latest measurement to assess carotid elasticity by vascular stiffness. CCS, which is adjusted circumference strain by pulse pressure, has higher feasibility and reliability showing more consistent result than existing parameters such as Peterson's elastic modulus and β index etc [14-16].

Arterial elasticity is considerably related with carotid intima-media thickness or plaque[17]. As DJS increased CCS, it could contribute to prevention of structural as well as functional degeneration like atherosclerosis and could be substituted treatment to subclinical atherosclerosis.

Usage of statins is recommended by international guidelines to treat and prevent carotid atherosclerosis because of its anti-inflammatory effect and inhibition on formation of atherosclerosis plaque.[18] However, because statins have shown adverse effects such as liver dysfunction and rhabdomyolysis[19], herbal medicine has drawn attention recently. Chunghyul-dan[20], Geiji-Bokryung-Hwan[21], Cardiotonic Pills[22] were reported to enhance blood circulation that recommended as alternative medicine for statins.

Danggui-jakyak-san, firstly mentioned in "Synopsis of Prescriptions of the Golden Chamber, GeumGueyoryak", could be divided into two groups according to its function: a blood associated herbs (Angelica gigas, Paeonia lactiflora and Cnidium officinale) and a water associated herbs (Atractylodes lancea, Alisma canaliculatum and Poria cocos)[6, 23]. In the blood-associated group, Angelica gigas and Paeonia lactiflora could nourish blood and stabilize 'Yin', whereas Cnidium officinale could relieve

depressed liver. Especially, Cnidium officinale with Angelica gigas can promote blood circulation[24]. As the water associated group can eliminate dampness by invigorating spleen[23], DJS could make blood and water circulation smoothly. There was a previous study that showed even acute administration of herbal medicine which can alleviate 'blood stagnation', could decrease central arterail blood pressure[25].

DJS-ethanol extract inhibited platelet aggregation which plays a patho-physiological role in thromboembolic disorders. This platelet aggregation and thrombus formation could be resulted from phosphoinositide breakdown and thromboxane A2 (TXA2) formation, inducing cerebrovasular disease and atherosclerosis[6, 7]. Especially platelet aggregation which induced by Adenosine 5′−diphosphate (ADP), Arachidonic acid sodium salt (AA) and collagen activation was well inhibited by Paeoniae Radix and Angelica Radix[26].

DJS was also reported to enhance estradiol level which is kind of estrogen and has the strongest biological activity[27]. As estrogen makes high density lipoprotein (HDL) higher and low density lipoprotein (LDL) lower, it can prevent aggravation of endothelial dysfunction such as atherosclerosis[28].

There were studies not only about inhibition on excessive platelet and thrombus activation but antioxidant effects of DJS that lead to antithrombotic properties by increase of platelet NO release and radical scavenging activity. NO is a main vasodilator involved in the maintaining normal vascular tone[29, 30]. Oxidative stress is closely related to the bioavailability of NO, as free radicals can directly search NO and disassemble its production enzyme, NO synthase (NOS)[30]. In endothelial dysfunction, the bioavailability of NO is compromised, resulting in increased vascular tone and resistance[31].

DJS could induce concentration-dependent increase of NO[32]. NO diffuses immediately into adjust smooth muscle and directly contact with platelet, acivates guanyl cyclase to produce cyclic guanosine monophosphate (cGMT). This procedure makes vessel wall dilated and prevent platelet activation and adhesion and intimal hyperplasia. In atherosclerosis, the protective action of nitric oxide is impaired and platelet accumulation occurs during the initial process of plaque formation. As NO restrains secretion of mitogen which is produced by platelets, inhibiting NO synthesis in blood vessels could delay plaque formation clearly[33].

On the contrary, DJS also induce decrease NO concentration-dependently if excessive NO is detected. This can be caused by decrease in inducible nitric oxide synthethase (iNOS) gene expression[32]. Excessive NO can be very toxic to cells and may generate other free radicals that can modify protein thiol groups, uncouple electron transport, and damage purine and pyrimidine bases[34]. Synthetically, DJS could maintain proper status of NO in body resulting in suppression of platelet aggregation, thrombus formation and finally, occurrence of atherosclerosis.

Previously DJS was used in studies about most of gynecological diseases, this study made DJS efficacy wider range to disease related blood circulation like cardiovascular disease or atherosclerosis.

Before this study, other studies reported that Cardiotonic Pills (CP, Tasly Pharmaceuticals Inc.) and Geiji-Bokryung-Hwan (GBH, Tsumura) made CCS improvement with similar protocol. These medicines traditionally used to blood stasis syndrome, which means pathological status formed by congestion of blood flow in body[21]. As DJS is also representative prescription to treat blood stasis syndrome, this can imply DJS made more solid base that blood-stasis related korean herbal medicine could improve blood circulation as well as vascular

endothelial wall function. These findings might be explained by provoked NO activation and inhibited platelet aggregation and adhesion.

This pilot study, however, has limitation because of absence in placebo medicine that result in absence of allocation concealment and double-blind test. Also, to find out maintenance period and long-term effect of DJS, further studies which set up longer period of study time are needed. Furthermore, studies that set up subject who has vascular endothelial dysfunction or potential risk factor of cardio/cerebrovascular disease like dyslipidemia, diabetes mellitus or hypertension, may be helpful.

IV. Conclusions

The present study has shown that Dangguijakyak-san improved arterial elasticity by common carotid circumference strain in healthy male subjects over time while other variables showed no significant change during study.

These results indicate DJS can enhance arterial endothelium function and be used to prevent blood wall related disease like atherosclerosis.

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