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Effects of Toki-shakuyaku-san on microcirculation of bulbar conjunctiva and hemorheological factors in patients with asymptomatic cerebral infarction

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In this study, the effects of Toki-shakuyaku-san on the microcirculation of bulbar conjunctiva in 11 patients with asymptomatic cerebral infarction were investigated with a video-microscopic system. After the administration of Toki-shakuyaku-san for four weeks, the flow volume rates of microcirculatory flow of the bulbar conjunctiva were increased ($p<0.05$). Hemorheological factors such as whole blood viscosity, plasma viscosity, and erythrocyte deformability were examined. Toki-shakuyaku-san improved whole blood viscosity and erythrocyte deformability ($p<0.05$), and plasma lipid peroxides decreased. These results suggested that the favorable effects of Toki-shakuyaku-san on cerebrovascular disorders take place via changes in microcirculatory flow, with the mechanisms being considered to be improvements in hemorheological factors and the anti-oxidant effect of Toki-shakuyaku-san.

Key words Toki-shakuyaku-san, microcirculation, asymptomatic cerebral infarction, whole blood viscosity, erythrocyte deformability, anti-oxidant effect.

Introduction

Toki-shakuyaku-san (TSS: Dang-gui-shao-yao-san), one of the famous traditional Chinese prescriptions, has been used to treat obstetric and gynecologic disorders since olden times. TSS is presently also used to treat sterility, anemia at pregnancy, hypopituitarism and menopausal syndrome. Further, TSS was reported to exert an effect against Alzheimer type dementia[1] and cerebrovascular dementia,[2] and thus is often used in a clinical setting.

Its mechanisms were reported to affect neuroendocrinology, such as stimulating the synthesis of acetylcholine receptor and increasing the kinds of catecholamine.[3] However, there are few reports concerning TSS and brain blood flow and hemorheology clinically. It is reported that asymptomatic cerebral infarction progresses to vascular dementia and cerebral infarction.[4,5] A decrease in cerebral blood flow due to endothelial dysfunction from aging and hypertension are thought to be the causative factors.[6]

In this study, we investigated the effect of TSS on the microcirculation and hemorheological factors in patients with asymptomatic cerebral infarction.

Subjects and Methods

Patients. Eleven patients without neurological disorder were diagnosed as asymptomatic cerebral infarction based on high-intensity lesions greater than 3 mm in size on T_2-weighted images coinciding with low-intensity lesions on T_1-weighted on MRI. They visited the Department of Japanese Oriental (Kampo) Medicine, Toyama Medical and Pharmaceutical University Hospital between October 2001 and March 2002. The group consisted of 5 males and 6 females, aged 50-72 years (mean ± S.D.). Informed consent was obtained from all patients. Their complications included 5 cases of hypertension, 3 cases of diabetes mellitus and 2 cases of hyperlipidemia. Although some of them were being treated by Western medicines that influenced hemorheological factors, these medicines were not changed from three months before entry into this study until the end of the four-week TSS administration.

Preparation of Toki-shakuyaku-san. TSS was prepared as a hot decoction. It consisted of 6.0g of Alismatis Rhizoma (*Alisma orientale* JUZEPCZUK), 5.0g of Paeoniae Radix (*Paeonia lactiflora* PALLAS), 4.0g of Atractylodis Rhizoma (*Atractylodes lancea* DE CANDOLLE), 4.0g of Hoelen (*Poria cocos* WOLF), 3.0g of Cnidii Rhizoma (*Cnidium officinale* MAKINO), and 3.0g of Angelicae Radix (*Angelica acutiloba* KITAGAWA). These crude drugs were obtained from Tochimoto Tenkaido Co. Ltd.(Osaka, Japan). These pharmacons were boiled in 600ml water for 40 minutes, and 300ml of hot decoction was formed. Patients were orally given the Toki-shakuyaku-san decoction three times a day (300ml/day) for four weeks.

Study protocol. Blood pressure was measured and microcirculation of bulbar conjunctiva was observed by video-microscopic system at about 9:00 a.m. after overnight fasting both just before and right after the four-week period of TSS administration. At the same time, blood was withdrawn from the cubital vein and anti-coagulated in EDTA-2Na (1.5mg/ml) to measure whole blood viscosity, plasma

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viscosity, erythrocyte deformability, red blood cell (RBC), hemoglobin (Hb), hematocrit (Ht), lipid peroxides (LPO), NO_2^-/NO_3^- and fibrinogen.

Measurement of microcirculatory flow. We used a video-microscope system to observe the venules of the bulbar conjunctiva.[7] The internal diameter (ID) of the vessels (their initial ID was about 18μm and they were mostly straight-line) was measured. The traveling distance of one erythrocyte during one second was measured frame by frame and the averaged values were calculated after three estimations as the flow velocity (Fve; μm/sec). Flow volume rate (Fvo; μm^3/sec) was obtained from the equation $Fvo=(1/2\ ID)^2 \times \pi \times Fve$.

Measurement of viscosity. The details of blood viscosity measurement were explained in our previous paper.[8] Whole blood was measured by coneplate rotational viscometer (Bio-rheolizer, Tokyo Keiki Co., Ltd., Tokyo, Japan) at five different points of shear rates (γ) (19.2, 38.4, 76.4, 192.0, 384.0 sec^{-1}) five times, respectively, and the averages of the five values were calculated. Using the remaining blood samples, this procedure was repeated. Final viscosity was estimated by the average at each point through the average of two repeated tests. Plasma viscosity was estimated at one shear rate (384.0 sec^{-1}) through the average of five values. All viscosity measurements were performed at a constant temperature of 37℃.

Measurement of erythrocyte deformability. The apparatus and sample preparation for measurement of erythrocyte deformability were described in our previous paper.[9] After high-speed centrifugation, plasma and buffy coat were removed. The remaining packed erythrocytes were washed three times with isotonic phosphate buffer (PBS; NaCl 93.4 mM, Na_2HPO_4 - NaH_2PO_4 3.2 mM, KCl 5.0 mM, glucose 5 mM, PH7.4, 295 mOsm/kg) and resuspended in isotonic PBS to a final concentration of 15%. Erythrocyte deformability was determined by measuring the filtration time required for 400μl of 15% red cell suspension to pass through a 5μm pore filter (Nucleopore, Costar Co., Ltd., CA, USA) under constant -10cm H_2O pressure. Erythrocyte deformability was calculated as the average of six repeated tests.

RBC, Hb, Ht, lipid peroxide and fibrinogen measurement. RBC, Hb and Ht were determined by automatic counter (Celltac α, MEK-6158, NIHON KOHDEN Co., Tokyo). LPO was measured by the Yagi method,[10] and plasma fibrinogen by the thrombin time method.[11]

NO measurement. NO is an extremely unstable molecule and rapidly undergoes oxidative degradation to stable inorganic nitrogen oxides NO_2^-/NO_3^- that were used here as indices of in vivo NO generation. Serum NO_2^-/NO_3^- was measured with an automated system (ENO-10; EICOM Co., Kyoto, Japan), based on the Griess reaction method.

Statistical analysis. Data were presented as mean ± standard error. Statistical comparisons were made using the Wilcoxon's t-test. The level of statistical significance was defined as $p<0.05$.

Results

There were no significant differences in blood pressure between before and after the four-week period of TSS administration. As for microcirculatory flow, the mean internal diameter of vessels and flow velocity did not change from before to after the four-week period of TSS administration, but the mean flow volume rate significantly increased from $0.9\pm0.09\times10^5$ μm^3/sec to $1.14\pm0.09\times10^5$ μm^3/sec (Table 1).

Whole blood viscosity was corrected by hematocrit at 45%. There were no significant differences between before and after the four-week period of TSS administration at low shear stress in whole blood viscosity. However, there was significant decrease in the viscosity at high shear stress from 4.14 ± 0.06 cp to 4.01 ± 0.09 cp. Plasma viscosity showed no significant difference at either low or high shear stress. Mean erythrocyte deformability significantly improved from 13.53 ± 0.35 msec to 12.91 ± 0.42 msec in the four-week period (Table 2). The number of RBC increased from $459.8\pm9.3\times10^4$/μl to $473.2\pm9.3\times10^4$/μl, Hb increased from 13.3 ± 0.3 g/dl to 14.1 ± 0.4 g/dl, and Ht increased from 43.4 ± 1.0 % to 44.9 ± 1.0 %, all significantly. LPO was significantly decreased from 2.69 ± 0.32 nmol/ml before, to 2.18 ± 0.16 nmol/ml after the four-week period of TSS administration. NO_2^-/NO_3^- and fibrinogen did not change in the four weeks (Table 3).

Table 1 Changes in blood pressure and microcirculatory flow

		Pre-administration	Post-administration
Blood pressure			
Systolic pressure	(mmHg)	132±6	136±5
Diastolic pressure	(mmHg)	81±4	82±4
Microcirculatory flow			
Internal diameter of vessels	(μm)	18.1±0.7	19.2±0.7
Blood flow velocity	(μm/sec)	353.3±31.6	389.5±16.2
Blood flow volume	($\times10^5\mu m^3$/sec)	0.9±0.09	1.14±0.09*

The results were expressed as mean ± S.E., n=11. Asterisks indicate significant differences from pre-administration (*: $p<0.05$).

Table 2 Changes in hemorheological factors

		Pre-administration	Post-administration
Corrected whole blood viscosity			
Low shear stress	(cp)	7.02±0.15	6.91±0.28
High shear stress	(cp)	4.14±0.06	4.01±0.09*
Plasma viscosity			
Low shear stress	(cp)	7.52±0.64	7.49±0.83
High shear stress	(cp)	1.52±0.05	1.41±0.03
Erythrocyte deformability	(msec)	13.53±0.35	12.91±0.42*

The results were expressed as mean ± S.E., n=11. Asterisks indicate significant differences from pre-administration (*: $p<0.05$).

Table 3 Changes in RBC count, plasma lipid peroxides, fibrinogen and No_2^-/No_3^-

		Pre-administration	Post-administration
Red blood cell	($\times 10^4/\mu l$)	459.8±9.3	473.2±9.3*
Hemoglobin	(g/dl)	13.3±0.3	14.1±0.4*
Hematocrit	(%)	43.4±1.0	44.9±1.0*
Lipid peroxides	(nmol/l)	2.69±0.32	2.08±0.16*
No_2^-/No_3^-	($10^{-5}M$)	25.0±6.4	25.5±4.8
Fibrinogen	(mg/ml)	184.6±9.5	196.6±11.9

The results were expressed as mean ± S.E., n=11. Asterisks indicate significant differences from pre-administration (*: $p<0.05$).

Discussion

Asymptomatic cerebral infarction is a significant disease, as it progresses to vascular dementia and cerebral infarction.[4,5] The mechanism underlying its cause is considered to be decreasing cerebral blood flow from endothelial dysfunction resulting from both aging and hypertension.[6] For its treatment, anticoagulants are not commonly used because of the possibility of inducing cerebral bleeding. This means that, under such circumstances, only hypertension receives treatment. In traditional Oriental medicine, there is the concept of "Oketsu", which means blood stasis. There are some prescriptions that are called "anti-Oketsu" drugs for treating blood stasis. TSS is one of these "anti-Oketsu" drugs, and it is used for patients whose health is adversely affected by coldness. It is also often used for gynecologic disease with anemia.

In this study, TSS had no effect on blood pressure, but the blood flow volume of the microcirculation at conjunctive bulbi increased significantly. One of its mechanisms was thought to be the improvement of erythrocyte deformability, and it was supported that the whole blood viscosity at high shear stress and the filtration time of RBC decreased. Further, LPO is an index of radical production, and an increase in production is a contributing factor in arteriosclerosis[12] and also weakens the NO effect, worsening blood circulation.[13] In the present study, plasma LPO decreased significantly, and therefore TSS was thought to have a favorable effect on blood circulation.

There was a slight increase in the number of RBC. It has also been reported that TSS had a hematopoietic effect and improved anemia. But as polycythemia is one of the risk factors of cerebral infarction, TSS is particularly useful for anemic patients.

Until now we have been studying the effects of Kampo formulations such as Keishi-bukuryo-gan[14] and Choto-san.[8] Comparing their results with the present result, TSS, Keishi-bukuryo-gan and Choto-san all improved microcirculation. TSS and Keishi-bukuryo-gan decreased whole blood viscosity, and TSS and Choto-san improved erythrocyte deformability. Furthermore, Keishi-bukuryo-gan decreased the levels of Ht and fibrinogen, TSS increased the RBC count, and Choto-san decreased the level of total cholesterol. Thus, each formula has its particular features, and they share that of the improvement of the microcirculation.

Generally, the use of Kampo formulas is still determined on the basis of traditional Oriental thinking. However, from the present study, it was possible to select the best formula not only by the method of traditional Oriental thinking but also by taking into account Western medical laboratory data.

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Japanese abstract

当帰芍薬散は陰虚証に用いられる代表的な駆瘀血剤である。今回，無症候性脳梗塞患者に当帰芍薬散を投与し血液レオロジー因子等に及ぼす影響を検討した。対象は富山医科薬科大学附属病院和漢診療科外来通院中で頭部MRI上，無症候性脳梗塞と診断した患者11名（男性5名，女性6名，平均年齢59.5±8.2歳）である。方法は，当帰芍薬散投与前と4週後に，ビデオ顕微鏡システムを用い，眼球結膜微小血管を観察し，血管内径，血流速度，血流量を測定した。また，採血により血液粘度，赤血球変形能，過酸化脂質，一酸化窒素代謝物，フィブリノーゲン，赤血球数，Hb，Ht値等を測定した。結果は，当帰芍薬散の4週間の投与により，血圧には変化を認めなかったが，眼球結膜微小血管の血流量が増加し，血液レオロジー因子の全血粘度と赤血球変形能の改善を認めた。また，Hb，Ht値の上昇と過酸化脂質値の低下を認めた。

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