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Effects of Choto-san on microcirculation of the bulbar conjunctiva, hemorheological factors and vascular function in patients with asymptomatic cerebral infarction and spontaneously hypertensive rats

Qiao YANG*

Department of Traditional Chinese Medicine, General Hospital, Tianjin Medical University, 154 Anshan Road, Heping-Qu, Tiangin-Shi, China, 300052.

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Abstract

Choto-san is a formula used for the treatment of headache and vertigo. Recently it has often also been used for hypertension and dementia. One of the mechanisms involved is thought to be the improvement of blood circulation, but the details are still unclear. In this study, at first we designed a clinical study to determine the effects of Choto-san on microcirculation and hemorhelolgical factors in patients with asymptomatic cerebral infarction, then we studied nitric oxide (NO) function, hemorheological factors and endothelial function in stroke-prone spontaneously hypertensive rats (SHR-SP).

In the clinical study, the effects of Choto-san on the microcirculation of bulbar conjunctiva were investigated with a video-microscopic system. The internal diameter of vessels, flow velocity and flow volume rate were examined and were found to have increased. Erythrocyte aggregability, evaluated by measuring the maximum diameter of a column of intravascular erythrocyte aggregation, was also improved. Simultaneously, hemorheological factors were examined and Choto-san improved the deformability of both erythrocytes and leukocytes, but not blood viscosity.

In the animal study, rats were given Choto-san in drinking water for eight weeks. Body weight, blood pressure, serum NO_2^-/NO_3^-, lipid peroxides, blood viscosity, erythrocyte deformability and endothelium-dependent/-independent relaxation were measured. The results indicated that Choto-san caused a decrease in blood pressure and an increase in erythrocyte deformability and NO function. Blood viscosity was not changed. Furthermore, endothelium-dependent relaxation by acetylcholine was significantly increased as compared to control.

These results suggest that Choto-san had favorable effects on cerebrovascular disorders and showed a protective effect on the endothelium, against cerebral vascular injury in the susceptible rat.

Key words Choto-san, asymptomatic cerebral infarction, spontaneously hypertensive rat, hemorheological factors, lipid peroxide, NO_2^-/NO_3^-.

Introduction

Choto-san is a formula used since ancient times for the care of headache and vertigo,[1] and it has become quite popular of late to administer it to relatively aged patients suffering from hypertension,[2] Alzheimer disease[3] and tinnitus.[4] Furthermore, in a recent doubled-blind, placebo-controlled study, we demonstrated that Choto-san was effective for vascular dementia.[5] However, the mechanism of the drug action with respect to improving cerebrovascular disorders is still uncertain. It was suggested that the improvement of cerebral blood flow was the mechanism involved in these favorable effects. As for reports concerning the improvement of blood circulation by Choto-san, Uncariae Ramulus et Uncus, the main component of Choto-san, has a hypotensive effect[6] and endothelium-dependent and -independent vasodilator effects.[7]

Since Eisenberg *et al.*[8] demonstrated that the increase in blood viscosity plays a part in causing cerebral infarction, abnormal hemorheological factors in cerebro-

*To whom correspondence should be addressed. e-mail : yang_qiaoqiao@hotmail.com

vascular disorders have attracted increasing attention. Several studies have proposed that increase of blood viscosity, acceleration of erythrocyte and platelet aggregation, decrease of erythrocyte and leukocyte deformability and elevation of fibrinogen concentration were noticed in patients with ischemic cerebrovascular damage.[9] In our previous paper, we reported the short-term effect of Choto-san on the microcirculation of bulbar conjunctiva in twelve healthy volunteers. The internal diameter of vessels, flow velocity and flow volume rate increased one hour after the oral administration of Choto-san.[10] These results suggest that Choto-san may possibly contribute to the microcirculatory regulation of the brain in patients with cerebrovascular disorders.

Choto-san is composed of 11 kinds of medical herbs, and there are thought to be several mechanisms involved in the improvement of blood circulation. The present study was designed in a clinical study to determine the effects of Choto-san on microcirculation and hemorheological factors in patients with asymptomatic cerebral infarction. As the next step, we examined NO function, hemorheological factors and endothelial function in stroke-prone spontaneously hypertensive rats (SHR-SP), which is a model of decreased cerebral circulation.

Clinical study

Subjects and Methods

Patients: The subjects were sixteen patients with asymptomatic cerebral infarction who visited the Department of Japanese Oriental (Kampo) Medicine, Toyama Medical and Pharmaceutical University Hospital. They consisted of 4 males and 12 females, aged 63.5±8.1 years (mean±S.D.), and their diagnosis was reached by magnetic resonance imaging. Informed consent was obtained from each patient. Although some of them were being treated by Western medicines that influenced hemorheological factors, such medicines had not been changed from three months before entry into this study until the end of four weeks of Choto-san administration.

Substances: Choto-san used in this study was prepared as hot infusion. It consisted of 5.0 g of Sekko (石膏), Gypsum Fibrosum, $CaSO_4 2H_2O$, 3.0 g of Kikka (菊花), Chrysanthemi Flos, *Chrysanthemum morifolium* RAMATULLE, 3.0 g of Choto (釣藤), Uncariae Uncis Cum Ramulus, *Uncaria sinensis* OLIVER, 3.0 g of Chimpi (陳皮), Aurantii Nobilis Pericarpium, *Citrus unshiu* MARKOVICH, 3.0 g of Ninjin (人参), Ginseng Radix, *Panax ginseng* C.A.MEYER, 3.0 g of Bakumonto (麦門冬), Ophiopgonis Tuber, *Ophiopogon japonicus* KER-GAWLER, 3.0 g of Bofu (防風), Saposhnikoviae Radix, *Saposhnikovia divaricata* SCHISCHKIN, 3.0 g of Bukuryo (茯苓), Hoelen, *Poria cocos* WOLF, 3.0 g of Hange (半夏), Pinelliae Tuber, *Pinellia ternata* BREITENBACH, 1.0 g of Kanzo (甘草), Glycyrrhizae Radix, *Glycyrrhiza uralensis* FISHER, and 1.0 g of Shokyo (生姜), Zingiberis Rhizoma, *Zingiber officinale* ROSCOE. These pharmacons were suspended in 600 ml of water, boiled for 30 to 40 minutes, and 300 ml of infusion solution was formed. Patients were orally given the Choto-san infusion three times a day (300 ml/day) for four weeks.

Study protocol: Before and after the four-week period of Choto-san administration, blood pressure and heart rate were measured and microcirculation of bulbar conjunctiva was observed by video-microscopic system[11] at about 9:00 a.m. after overnight fasting. At the same time, 21 ml of blood was withdrawn from the cubital vein, anticoagulated in EDTA-2Na (1.5mg/ml) to measure the hemorheological parameters of whole blood viscosity, plasma viscosity, erythrocyte deformability and leukocyte deformability.

Measurement of microcirculatory flow: We observed the venules of the bulbar conjunctiva of internal diameter of about 20 μm, which were mostly straight-line, and used a video-microscope system.[12] The internal diameter of the vessels (ID; μm) 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 erythrocyte aggregability: The maximum diameter of a column of intravascular erythrocyte aggregation (DEA) was defined as the maximum diameter of the largest venule in which intravascular erythrocyte aggregation in the pleural venules of the bulbar conjunctiva was observed by the video-microscope system. In a previous report, we showed that DEA served as a useful index for evaluating erythrocyte aggregability *in vivo*.[13]

Measurement of viscosity: The details were explained in our previous paper.[14] Whole blood and separated plasma were measured by coneplate rotational viscometer (Bio-rheolizer, Tokyo Keiki Co., Ltd., Tokyo). Whole blood viscosity was measured 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 sample, this procedure was repeated. The final viscosity was estimated for each point through the average of two repeated tests. The plasma viscosity was estimated at one shear rate (384.0 sec^{-1}) through the average of five values.

Measurement of erythrocyte deformability: The apparatus and sample preparation for measurement of erythrocyte deformability were described in our previous paper.[15] After high-speed centrifugation, plasma and buffy coat were removed. The remaining packed erythrocytes were washed three times with isotonic phosphate buffer (PBS) (pH=7.4, 295mOsm/kg) and resuspended in isotonic PBS to a final concentration of 15%. Erythrocyte deformability was determined by measuring the filtration time required for 400 μl of 15% red cell suspension to pass through a 5 μm pore filter (Nucleopore, Costar Co., Ltd., USA) under constant -10cm H_2O pressure. The erythrocyte deformability was calculated as the average of six repeated tests.

Measurement of leukocyte deformability : To determine leukocyte deformability, at first, whole blood suspension and correlative red cell suspension were prepared, respectively.[16] Whole blood suspension prepared from the whole blood sample was diluted with autologous plasma to leukocyte counts of 3000/ μl and hematocrit was measured. Similar to the measurement of erythrocyte deformability, erythrocytes were separated and washed, and only the third washing was done at high-speed centrifugation to obtain thick density. Concentrated erythrocytes from the middle part of the erythrocyte column were aspirated and added to autologous plasma, and its hematocrit was adjusted to plasma-diluted whole blood of leukocyte counts of 3000/ μl. By the same process as for the erythrocyte deformability, the filtration time for the respective cell suspensions was calculated and the disparity of the respective averages was defined as leukocyte deformability.

Statistical analysis : The 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

Figure 1 shows the changes in microcirculatory flow in the bulbar conjunctiva after the administration of Choto-san for four weeks. The mean (±S.E.) internal diameter significantly increased from 21.0±0.8 μm at pre-administration to 22.4±0.7 μm at post-administration (Fig.1a). The mean flow velocity significantly increased from 327±28 μm/sec to 390±36 μm/sec (Fig.1b). The

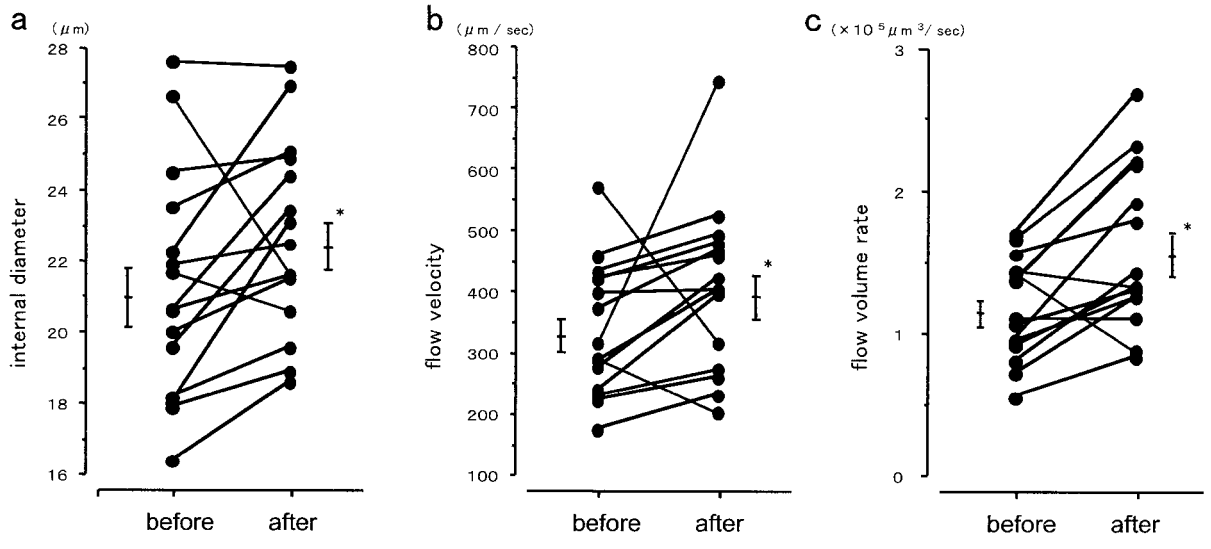

Fig. 1 Changes in internal diameter (a), flow velocity (b), flow volume rate (c) in bulbar conjunctiva following the oral administration of Choto-san. Data expressed as mean±S.E. *: $p<0.05$ vs. before administration.

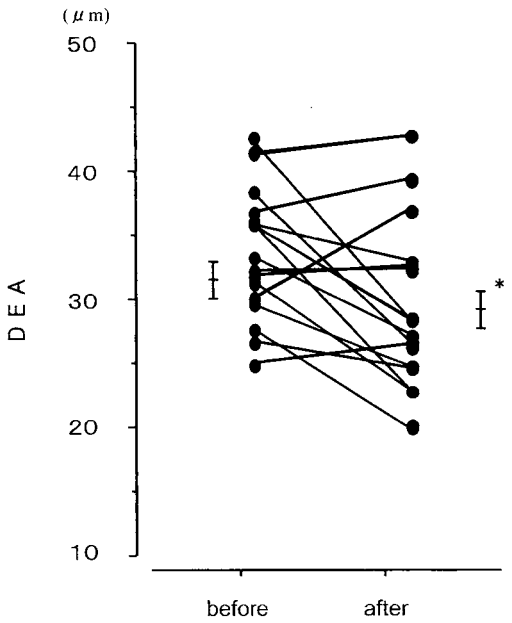

Fig. 2 Changes in DEA in bulbar conjunctiva following the oral administration of Choto-san. Data expressed as mean±S.E. * : $p<0.05$ vs. before administration.

Table I Changes in blood viscosity following the administration of Choto-san

		pre-administration	post-administration	
Corrected whole blood viscosity				
low shear stress	(cp)	7.52±064	7.49±0.83	NS
high shear stress	(cp)	4.26±0.23	4.31±0.22	NS
Plasma viscosity	(cp)	1.48±0.08	1.54±0.16	NS

Each value is mean±S.D. n = 16. NS : not significant

mean flow volume rate significantly increased from 1.11 ± 0.09 × 10^5 μ m^3/sec to 1.52 ± 0.14 × 10^5 μ m^3/sec (Fig.1c).

The mean DEA significantly decreased from 32.0 ±1.3 μm at pre-administration to 27.9±1.6 μm at 4 weeks after the administration of Choto-san (Fig.2).

Table I shows the changes of blood viscosity. Whole blood viscosity was corrected by hematocrit at 45%. There were no significant differences between pre- and post-administration in whole blood viscosity (low shear stress and high shear stress) and plasma viscosity.

Changes of erythrocyte deformability and leukocyte deformability are shown in Figure 3. Mean erythrocyte deformability significantly improved from 12.7 ± 0.5 msec at pre-administration to 11.7±0.3 msec at 4 weeks after the administration of Choto-san (Fig.3a). Mean leukocyte deformability significantly improved from 2.6± 0.3 msec to 1.9±0.3 msec (Fig.3b).

Animal study

Materials and Methods

Animals: Sixteen 6-week-old male SHR-SP obtained from Sankyo Labo Service (Toyama, Japan) were used. They were kept in an animal room at an ambient temperature of 23±1℃ under a 12 h dark-light cycle. They were allowed an adaptation period of one week, during which they were fed a commercial feed (CE-2,

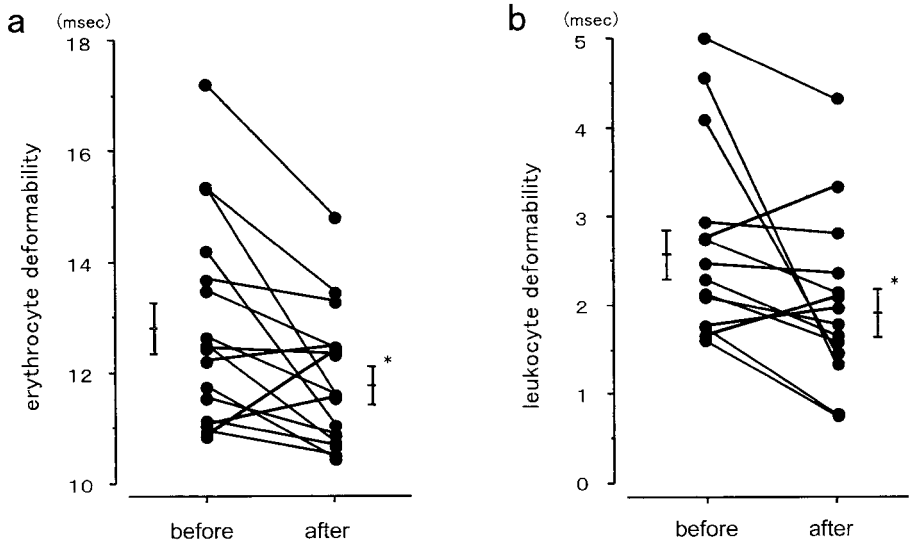

Fig.3 Changes in erythrocyte deformability (a) and leukocyte deformability (b) following the oral administration of Choto-san. Data expressed as mean±S.E. * : $p<0.05$ vs. before administration.

CLEA Japan Inc., Tokyo, Japan).

Experimental protocols met the "Guidelines for Animal Experimentation" approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society.

Drugs and treatment: The extract was composed of 11 kinds of medical herbs in the same ratio as the clinical study, and the extract was donated by Tsumura & Co. Three-dimensional HPLC profiles of this extract were demonstrated in our previous report.[5] Rats were randomly assigned to two groups. One group received distilled water, and the Choto-san group received 450 mg/Kg/day of Choto-san extract dissolved in distilled water for eight weeks. Blood samples were obtained from the baseline period and at sacrifice.

Body weight and blood pressure measurement: Body weight and blood pressured (monitored indirectly using the rail-cuff method) were measured at bi-weekly intervals from the baseline period until sacrifice.

Measurement of serum NO and lipid peroxides: NO is an extremely unstable molecule and rapidly undergoes oxidative degradation to stable inorganic nitrogen oxides NO_2^-/NO_3^-, which 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. Lipid peroxides were measured according to the method of Yagi.[17]

Measurement of blood viscosity: The details of blood viscosity measurement were explained in our previous study.[14] Whole blood viscosity was corrected by hematocrit at 45%, and 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. The averages of the five values were then calculated. Using the remaining blood samples, this procedure was repeated. The final viscosity was estimated for each point by the average of the two repeated tests. Plasma viscosity was estimated at the highest shear rate of 384.0 sec^{-1} by the average of five measurements. All measurements were performed at a constant temperature of 37℃.

Erythrocyte deformability: The apparatus and sample preparation for the measurement of erythrocyte deformability were described in our previous report.[15] After high-speed centrifugation, plasma and buffy coat were removed. The remaining packed erythrocytes were washed three times with isotonic phosphate buffer (PBS: NaCl 93.4mM, Na_2HPO_4 - NaH_2PO_4 3.2 mM, KCl 5.0 mM, glucose 5 mM, PH 7.4, 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 400 μl of 15% red cell suspension to pass through a 5 μm pore filter (Nucleopore, Costar Co.,Ltd., California, USA) under constant -10 cm H_2O pressure. Erythrocyte deformability was calculated as the average of six repeated tests.

Relaxation experiments: The rats were anesthetized (50 mg/kg i.p. pentobarbiturate) and killed by drawing blood from the heart. A section of the thoracic aorta was carefully cleaned of fat and connective tissues, and 3-mm ring preparations were made.

The rings were mounted on steel hooks in a Magnus chamber (Kishimoto UC-5TD, Kyoto, Japan). One end of the aorta was attached to a force-displacement transducer (Kishimoto UM-203) so that its isometric contraction could be recorded (Niko Bioscience T-634, Tokyo, Japan). Baths were filled with 5 ml of Krebs solution with the following composition (mM): NaCl 120, KCl 4.7, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 2.5 and glucose 10.0. The solution was maintained at 37℃ and bubbled continuously with 5% CO_2 in O_2 at pH 7.4.

The rings were equilibrated for 40 min at an initial resting tension of 1 g. During this time, the Krebs solution in the tissue bath was replaced every 15 min. The rings were then precontracted with 5×10^{-7} M noradrenaline (NA). For endothelium-dependent relaxations, vessels were relaxed with acetylcholine (10^{-9} to 10^{-4} mol/L). To study the direct relaxation of vascular smooth muscle, vessels were relaxed with sodium nitroprusside (10^{-9} to 10^{-4} mol/L). Relaxation was expressed as percentage of the decrease in maximal tension obtained by NA-induced contraction.

Statistical analysis: Data were presented as mean ± standard error. Statistical comparisons were made using the Mann-Whitney test and repeated measures ANOVA. The level of statistical significance was defined as $p<0.05$.

Results

Throughout the 8 weeks of Choto-san administra-

tion, body weights did not significantly differ between the two groups. However, systolic and mean blood pressure of the Choto-san group decreased significantly as compared to the control group ($p<0.05$). Also, in the Choto-san group, serum NO_2^-/ NO_3^- levels tended to increase, but serum lipid peroxide levels decreased significantly as compared to the control group ($p<0.05$) (Table II).

There were no significant differences between the control and Choto-san groups in whole blood viscosity (low and high shear stress) and plasma viscosity, but erythrocyte deformability of the Choto-san group significantly improved in comparison to the control group ($p<0.01$) (Table III).

Acetylcholine induced endothelium-dependent relaxation, reaching a maximum at 10^{-4}M, and relaxation of the Choto-san group was increased to a greater degree than the control group, with a statistically significant difference ($p<0.05$). Maximum relaxation was 59.8±5.1% and 41.5±6.5% in the Choto-san group and control, respectively (mean±S.E., n=8) (Fig. 4a). In endothelium-independent relaxation, there was no significant difference with sodium nitroprusside between the two groups. Maximum relaxation at 10^{-4}M was 95.1±0.5% and 97.0±0.6% in the Choto-san group and control, respectively (mean±S.E., n=8) (Fig.4b).

Table II Changes in the different experimental groups of SHR-SP

	0 weeks		8 weeks	
	control	Choto-san	control	Choto-san
Body weight (g)	144.5±3.9	146.0±4.1	259.5±8.9	265.5±6.8
Systolic pressure (mmHg)	152.8±6.2	154.6±4.8	243.1±4.7	214.9±4.7*
Mean pressure (mmHg)	128.2±5.6	126.4±7.2	202.5±6.6	179.6±7.2*
Serum NO_2^-/NO_3^- ($\times 10^{-5}$ M)	–	–	16.5±0.9	17.9±2.1
Lipid peroxide ($\times 10^{-6}$ M)	–	–	2.36±0.07	2.23±0.05*

Each value is mean ± S.E.. n=8 rats. *$p<0.05$ vs. control group.

Table III Changes in rheologic factors of SHR-SP

		control	Choto-san
Corrected whole blood viscosity			
low shear stress (19.2sec^{-1})	(cp)	9.11±0.80	8.83±0.50
high shear stress (384.0sec^{-1})	(cp)	4.32±0.25	4.13±0.14
Plasma viscosity	(cp)	1.04±0.20	1.37±0.03
Erythrocyte deformability	(msec)	6.16±0.29	5.28±0.14*

Each value is mean ± S.E.. n=8 rats. *$p<0.01$ vs. control group.

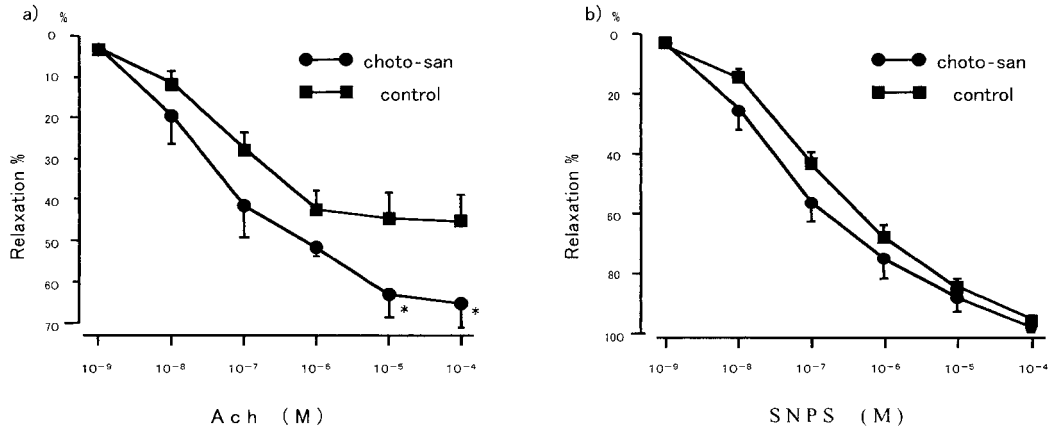

Fig.4 Graph showing a) endothelium-dependent relaxation in response to acetylcholine (ACH), b) endothelium-independent relaxation in response to sodium nitroprusside (SNP) in aortic rings of SHR-SP treated for 8 weeks. Choto-san was given at 300mg/kg/day solution in test group and distilled water was received in the control group during all the experiment period. Values were expressed as percentage of decrease in the maximal tension contracted with 5×10^{-7} M NA. Shown is mean±S.E. of 8 determinations in each group. Differernce between control and Choto-san in graph a) are statistically significant (*:$p<0.05$; n=8)

By 8 weeks, two rats of the control group had developed paralysis, and brain dissections revealed the presence of blood spots. None of the Choto-san group showed any signs of paralysis, and there were no blood spots on their brains.

Discussion

The Kampo formula Choto-san is now often used in a clinical setting. The underlying mechanisms of its actions are, however, still unclear. Regarding its main component, Uncariae Ramulus et Uncus, some pharmacological,[18] central nervous[19] and cardiovascular effects[20] have been reported. Ginseng Radix, another component of Choto-san, played a role in the improvement of hemorheological factors.[21]

Our previous study,[10] in which Choto-san was administered to healthy volunteers, demonstrated its improvement effects on blood flow in microvessels of the bulbar conjunctiva. In the present study, we examined the effects of Choto-san on the microcirculation of the bulbar conjunctiva in patients with asymptomatic cerebral infarction. Similar to the previous study, the results revealed increases in the internal diameter of vessels flow velocity and flow volume rate in the patients' bulbar conjunctiva.

In addition, we considered various hemorheological factors influencing microcirculatory disturbance such as the increase of blood viscosity, acceleration of erythrocyte aggregation, and decline of blood cell deformability. At the beginning of hemorheological studies, many researchers investigated blood viscosity, erythrocyte aggregation and erythrocyte deformability. More recently, researchers have become interested in abnormal rheological behaviors of leukocytes in the disturbance of microcirculation. It has been recognized that leukocytes are an important player in the microcirculatory environment because of their large size, poor deformability and easy activation. The decline of leukocyte deformability and the acceleration of leukocyte aggregation were closely concerned with the occurrence of cerebrovascular attacks.[22,23]

The present results showed that DEA as an index of erythrocyte aggregation, erythrocyte deformability and leukocyte deformability in patients with asymptomatic cerebral infarction were improved by administering Choto-san for four weeks. These findings indicate that Choto-san has the pharmacological activity to work against accelerated erythrocyte aggregation and to improve the deformability of blood cells, facilitating whole blood filterability through microvessels. Furthermore, Choto-san was shown to extend microvessels and to increase velocity and volume of blood flow in the patients' bulbar conjunctiva. Taken together, it is suggested that the pharmacological action of Choto-san may include favorable hemorheological effects on the cerebral microcirculation and provide a useful method for the treatment of cerebrovascular disorders.

SHR-SP is a cerebral stroke model of the hypertensive rat. One of the causes of cerebral stroke was reported to be endothelial dysfunction based on vascular necrosis due to decreased brain circulation and hypertension.[24] This prompted us to use this model to examine the vascular protection effect of Choto-san. It was found that the blood pressure of the Choto-san group was significantly decreased compared to the control group. As there was no change in body weight between the two groups, their growth was thought to be the same, and the decrease in blood pressure was attributed to the effect of Choto-san. There have been some reports on the hypotensive effect of Choto-san.[2] Concerning the mechanism for this, it is known that alkaloids of Uncariae Ramulus et Uncus have a calcium-antagonist effect,[25] but because of the very small amount involved, we decided to search for other factors.

The relation between hypertension and hemorheological disorder was reported.[26] We investigated the hemorheological factors in this study. Erythrocyte deformability showed an improvement in clinical and animal study, but there were no significant changes in whole blood viscosity and plasma viscosity. It had been reported that Keishi-bukuryo-gan (桂枝茯苓丸), another famous Kampo formula was known to improve blood circulation by improving hemorheological factors such as blood viscosity.[27] Hence the effect of Choto-san on hemorheology differs from that of Keishi-bukuryo-gan. As for the mechanism of the improvement of erythrocyte deformability, because calcium antagonist was reported to play a role in vasodilatation and the improvement of hemorheology,[28] it is possible that alkaloids contained in *Uncaria sinensis* have the same effect. However, blood viscosity is correlated with various

hemorheological factors, the improvement of erythrocyte deformability were not enough to exert an influence on blood viscosity. Moreover, it has recently been reported that infarct size and outcome depend on the extent of residual microvascular perfusion in cerebral ischemia and that improvement of blood cell filterability is more important than a reduction of blood viscosity.[29]

We also studied the effect of Choto-san on the endothelium and NO function, because the effects of nitric oxide and free radicals are closely related to vasomotion.[30] We have already reported that Uncariae Ramulus et Uncus has an endothelium-dependent vasodilatation effect and a suppressive effect on vasoconstriction by the action of free radicals.[31] NO originating in the endothelium not only has the effect of vasodilatation, but also of protecting platelets and white cells against adhesion to the endothelium[32,33] and inhibiting the proliferation of vascular smooth muscle cells.[34] Then we measured serum NO_2^-/ NO_3^- as NO, as well as lipid peroxides, as an index of free radicals at the same time. There was a tendency for serum NO_2^-/ NO_3^- in the Choto-san group to increase, and lipid peroxides were decreased significantly. NO was reported to become inert by free radicals,[35] and the decrease in free radicals in the Choto-san group was only a little, but the effect of NO on the endothelium was nevertheless possibly useful in the Choto-san group.

The effect of the endothelium-dependent relaxing on the thoracic aorta was examined and the Choto-san group was found to be superior to the control group. Of course, the function of the thoracic aorta is different from that of the cerebral artery. But considering the decrease in systemic blood pressure, improvement of hemorheological factors and the increase of NO function, Choto-san was thought to possess a synergistically protective effect for cerebral vessels. In fact, when removing the brains from both SHR-SP groups, we observed their surfaces and found that two of eight in the control group but none of eight in the Choto-san group had blood spots.

In humans, multiple cerebral small infarction is reported to be a vascular disease caused by hypertension and aging.[36] Regarding this point, SHR-SP serves as a useful model for human multiple cerebral infarction. In summary, the clinical and animal study provided that Choto-san has favorable effects on cerebrovascular disorders and the endothelium, exerts a protective effect against cerebral stroke in SHR-SP. NO side effects such as bleeding tendency and organic trouble with the extended use of Choto-san have been reported, so clinically Choto-san is thought to be useful against cerebral infarction, and long-term studies are eagerly awaited.

Acknowledgment

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和文抄録

脳血管障害患者を対象とする臨床研究とSHR-SPを用いる基礎研究を行い，微小循環血流や血液流動性を左右する血液粘度，血球変形能など血液レオロジー因子及び血管内皮機能について釣藤散の影響を検討した。無症候性脳梗塞患者の4週間服薬では，赤血球集合能の指標DEA，血管内径，血流速度，血流量が有意に改善した。血液粘度は改善しなかったが，赤血球及び白血球変形能は有意に改善した。SHR-SP雄16匹を用いた8週間釣藤散投与では，収縮期血圧と平均血圧は有意に低かった。血液粘度に変化はなかったが，赤血球変形能は釣藤散群が有意に改善した。また，釣藤散群では10^{-5}M Ach投与による内皮依存性弛緩作用が有意に強く認められた。血中NO_2^-/NO_3^-は両群間に有意差がなかった。血漿過酸化脂質は鉤藤散群が有意に低下していた。

釣藤散は微小循環の血流や血液レオロジー因子を改善することで，脳血管障害患者の脳血流障害を改善したものと考えられた。また，釣藤散の降圧作用や血管内皮保護作用などの関与で，SHR-SPの脳卒中発症を抑制した。本研究から，釣藤散が脳血管障害に対して多面的な作用を介して臨床効果を発現している可能性が考えられた。

*〒300052　中国天津市和平区鞍山道154号
中国天津医科大学附属総医院・中医科　楊　喬

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