Effects of Choto-san (Diao-Teng-San) on microcirculation of bulbar conjunctiva and hemorheological factors in patients with asymptomatic cerebral infarction

Qiao YANG[a], Toshiaki KITA[b], Hiroaki HIKIAMI[c], Yutaka SHIMADA[a], Takashi ITOH[b] and Katsutoshi TERASAWA*[a]

[a] *Department of Japanese Oriental (Kampo) Medicine, Faculty of Medicine,*
[b] *Department of Kampo Diagnostics, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University,*
[c] *Department of Japanese Oriental (Kampo) Medical Center, Suwa Central Hospital*

(*Received February 16, 1999. Accepted May 24, 1999.*)

Abstract

In this study, the effects of Choto-san (釣藤散) on the microcirculation of bulbar conjunctiva in 16 patients with asymptomatic cerebral infarction were investigated with a video-microscopic system. After the administration of Choto-san for four weeks, variables of microcirculatory flow of the bulbar conjunctiva, that is, the internal diameter of vessels, flow velocity and flow volume rate were increased ($p<0.05$). Erythrocyte aggregability, evaluated by measuring the maximum diameter of a column of intravascular erythrocyte aggregation, was also improved ($p<0.05$). Simultaneously, hemorheological factors such as whole blood viscosity, plasma viscosity, erythrocyte deformability and leukocyte deformability were examined. Choto-san improved deformability of both erythrocytes and leukocytes ($p<0.05$), but not blood viscosity. These results suggest that Choto-san may have favorable effects on cerebrovascular disorders through changes in microcirculatory flow, erythrocyte aggregability and blood cell deformability.

Key words asymptomatic cerebral infarction, Choto-san, Hemorheology, leukocyte deformability, Microcirculation.

Abbreviations Choto-san (Diao-Teng-San), 釣藤散 ; DEA, the maximum diameter of the column of intravascular erythrocyte aggregation ; FVe, flow velocity ; FVo, flow volume rate ; ID, internal diameter of the vessel.

Introduction

Choto-san (Diao-Teng-San ; 釣藤散) is a Kampo medicine administered to relatively aged patients suffering from hypertension, cerebrovascular disorders and such subjective symptoms as headache, dizziness, shoulder stiffness *etc.*[1] Furthermore, a recent double-blind, placebo-controlled study[2] demonstrated that Choto-san is effective in treating vascular dementia. However, the mechanism of the drug action with respect to improving cerebrovascular disorders is still uncertain.

We have recently 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.[3] These results suggest that Choto-san may possibly contribute to the microcirculatory regulation of the brain in patients with cerebrovascular disorders.

Since Eisenberg *et al.*[4] demonstrated that the increase in blood viscosity plays a part in causing

*〒930-0194 富山市杉谷2630
富山医科薬科大学医学部和漢診療学講座 寺澤捷年
2630 Sugitani, Toyama 930-0194, Japan

cerebral infarction, abnormal hemorheological factors in cerebrovascular disorders have attracted increasing attention. Several studies have proposed that increase of blood viscosity, acceleration of erythrocyte and platelet aggregation, decreases of erythrocyte and leukocyte deformability and elevation of fibrinogen concentration were noticed in patients with ischemic cerebrovascular damages.[5]

The present study was designed to determine the effects of Choto-san on the microcirculation and hemorheological factors in patients with asymptomatic cerebral infarction.

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 Bakumondo (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[6] 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 (1.5 mg/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.[7] 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*.[8]

Measurement of viscosity : The details were explained in our previous paper.[9] 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.[10] 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, 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., USA) under constant -10 cm 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.[11] 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 mean flow volume rate significantly increased from $1.11\pm0.09\times10^5\mu m^3$/sec to $1.52\pm0.14\times10^5\mu m^3$/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

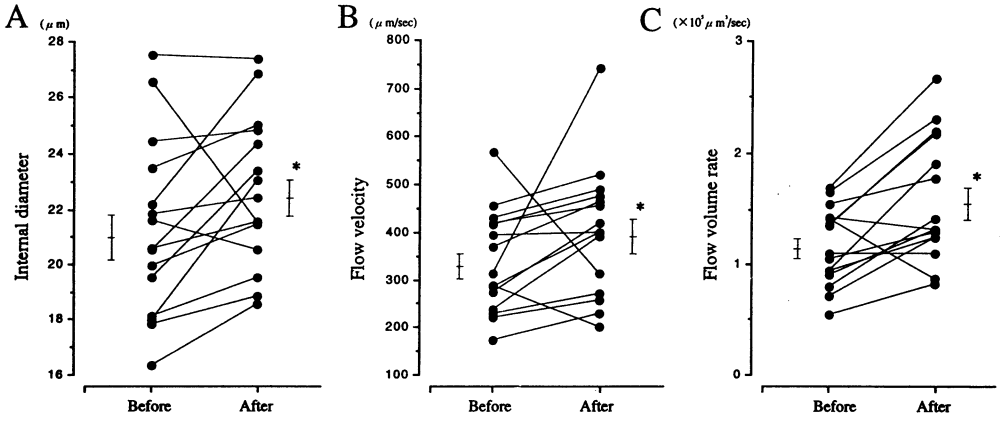

Fig. 1 Changes in internal diameter (A), flow velocity (B), flow volume rate (C) in bulbar conjunctiva following the oral administration of Choto-san in 15 patients with asymptomatic cerebral infarction. Data expressed as mean±S.E. *: $p<0.05$ vs. before administration.

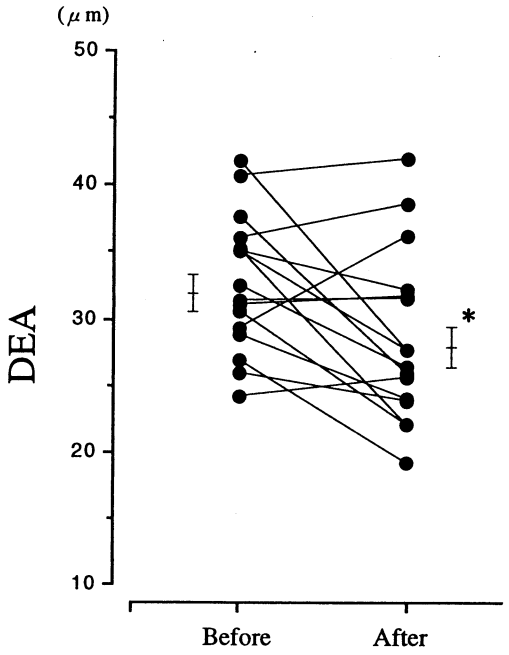

Fig. 2 Changes in DEA in bulbar conjunctiva following the oral administration of Choto-san in 16 patients with asymptomatic cerebral infarction. Data expressed as mean±S.E. *: $p<0.05$ vs. before administration.

(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).

Discussion

Our previous study, in which Choto-san was administered to healthy volunteers, demonstrated its improvement effects on blood flow in microvessels of the bulbar conjunctiva. However, it remained unknown whether the salutary effects may be gained by administering Choto-san to patients with microcirculatory disturbance. In the present study, we examined the effects of Choto-san on the microcirculation of the bulbar conjunctiva in patients with

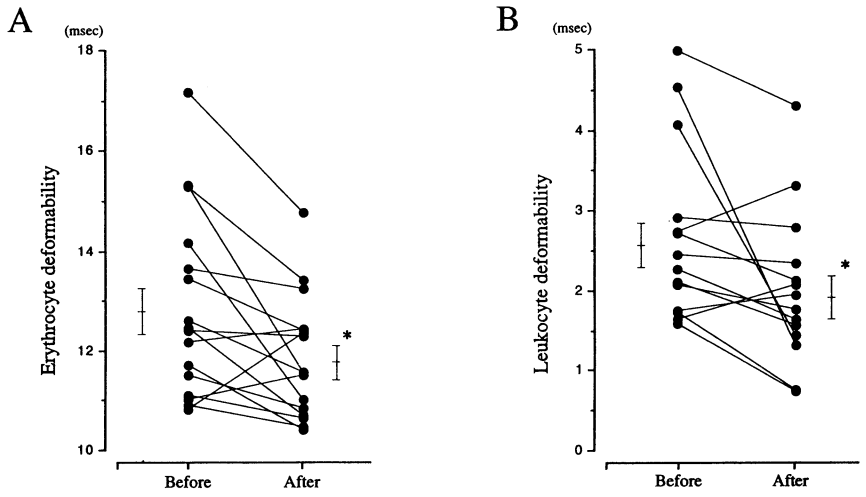

Fig. 3 Changes in erythrocyte deformability (A) and leukocyte deformability (B) following the oral administration of Choto-san in 16 patients with asymptomatic cerebral infarction. Data expressed as mean±S.E. *: $p<0.05$ vs. before administration.

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±0.64	7.49±0.83	N.S.
high sherar stress	(cp)	4.26±0.23	4.31±0.22	N.S
plasma viscosity	(cp)	1.48±0.08	1.54±0.16	N.S.

The results were expressed as mean±S.D., n=16. N.S.: not significant

asymptomatic cerebral infarction. Similar to the previous study [3] using healthy volunteers, 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. For example, it was reported that the decline of leukocyte deformability and the acceleration of leukocyte aggregation were closely concerned with the occurrence of cerebrovascular attacks.[12,13]

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.

In our previous studies, it had been reported that Keishi-bukuryo-gan (桂枝茯苓丸), one of the Kampo prescriptions, increased the flow velocity and flow volume rate of the microvessels in the bulbar conjunctiva, improved intra-vascular erythrocyte aggregation and erythrocyte deformability, and lowered blood viscosity in patients with cerebro-spinal vascular disease.[8,14] Several of these effects were similar to administration of Choto-san, however, in the present study, the vasodilative action of Choto-san on the bulbar microvessels was considered as an important pharmacologic action to improve microcirculation which was not shown by Keishi-bukuryo-gan.

In this study, the administration of Choto-san did not significantly change whole blood viscosity and plasma viscosity. It is well known that blood viscosity is correlated with erythrocyte factors (hematocrit, erythrocyte aggregability, erythrocyte deformability), leukocyte factor (leukocyte deformability), platelet factor (platelet aggregation), and plasma factors (fibrinogen, albumin, γ-globulin) *etc.* Although the Choto-san administration improved erythrocyte aggregability and blood cell deformability, these effects 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.[15]

Acknowledgments

This study was supported by a grant-in-aid for the Funds for Comprehensive Research on Aging and Health from the Japanese Ministry of Health and Welfare.

和文抄録

今回，無症候性脳梗塞患者16名を対象に，眼球結膜微小循環に及ぼす釣藤散の効果をビデオ顕微鏡システムを用いて検討した。釣藤散を4週間投与後，眼球結膜微小循環の血管内径，血流速度，血流量が増加した（$p<0.05$）。血管内赤血球集合現象を認める血管の最大内径で評価される赤血球集合能も改善した（$p<0.05$）。同時に，血液レオロジー因子である全血粘度，血漿粘度，赤血球変形能，白血球変形能も検討したところ，釣藤散は赤血球と白血球の変形能を改善したが（$p<0.05$），血液粘度は改善しなかった。以上の結果から，釣藤散は微小循環血流や赤血球集合能，血球変形能を改善することで，脳血管障害に好影響を与える可能性が示唆された。

References

1) Terasawa, K. : Kampo. Standard Mcintyre, Tokyo, pp.122-234, 1993.
2) Terasawa, K., *et al.* : Choto-san in the treatment of vascular dementia : a double-blind, placebo-controlled study. *Phytomedicine* **4**, 15-22, 1997.
3) Yang, Q., *et al.* : Effects of Choto-san on microcirculation of the bulbar conjunctiva and hemorheological factors in normal subjects. *J. Trad. Med.* **14**, 302-303, 1997.
4) Eisenberg, S. : Blood viscosity and fibrinogen concentration following cerebral infarction. *Circulation (supple II)* **33-34**, II-10-II-14, 1966.
5) Honda E. : Hemorheology in cerebral infarction. *Nippon Biorheology Gakkaishi* **10**, 23-36, 1996.
6) Tsushima, N., *et al.* : Human microcirculation by intravital videomicroscopic system. *Bibl. Anat.* **20**, 716-719, 1981.
7) Terasawa, K., *et al.* : The characteristics of the microcirculation of bulbar conjunctiva in "Oketsu" syndrome. *J. Med. Pharm. Soc. WAKAN-YAKU* **5**, 200-205, 1988.
8) Kohta, K., *et al.* : Effects of Keishi-bukuryo-gan on erythrocyte aggregability in patients with multiple old lacunar infarction. *J. Med. Pharm. Soc. WAKAN-YAKU* **10**, 251-259, 1993.
9) Terasawa, K., *et al.* : Rheological studies on "Oketsu" syndrome I. The blood viscosity and diagnostic criteria. *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 98-104, 1986.
10) Hikiami, H., *et al.* : Erythrocyte deformability in "Oketsu" syndrome and its relations to erythrocyte viscoelasticity. *J. Trad. Med.* **13**, 156-164, 1996.
11) Kikuchi, Y. : Effects of leukocytes and platelets on blood flow through parallel array of microchannels : Micro- and macroflow relation and rheological measures of leukocyte and platelet activities. *Microvascular Research* **50**, 288-300,1995.
12) Ernst, E., Matrai, A. and Paulsen, F. : Leukocyte rheology in recent stroke. *Stroke* **18**, 59-62, 1987.
13) Ciuffetti, G., *et al.* : Leukocyte rheology in the early stage of ischemic stroke. *Klin. Wochenschr.* **67**, 762-763, 1989.
14) Tosa, H., *et al.* : Effects of Keishi-bukuryo-gan on patients with cerebro-spinal vascular disease. *J. Med. Pharm. Soc. WAKAN-YAKU* **6**, 13-19, 1989.
15) Forconi, S., Pieragalli, D., Guerrini, M., *et al.* : Hemorheology and peripheral arterial diseases. *Clin. Hemorheol.* **7**, 145-158, 1987.