Original 和漢医薬学会誌 4, 20－25, 1987

Effects of Tôki-syakuyaku-san on blood viscosity and platelet functions in normal subjects

Kazuo TORIIZUKA,*[a] Zhang Tie ZHONG,[b] Katsutoshi TERASAWA,[a] Megumi OKAMOTO[a] and Hiroyori TOSA[a]

[a] *Department of Sino-Japanese (Kampoh) Medicine, Toyama Medical and Pharmaceutical University*
[b] *Department of Traditional Medicine, China-Japan Friendship Hospital*

(Received December 10, 1986. Accepted February 17, 1987.)

Abstract

Tôki-syakuyaku-san (Dang-Gui-Shao-Yao-San) is one of the anti-"oketsu" prescriptions of traditional Kampoh medicine. Effects of this prescription on blood viscosity and thromboxane synthesis in platelets were investigated in healthy volunteers (6 males and 6 females). They were administered 7.5 grams per day of Tôki-syakuyaku-san preparation for one week. Whole blood viscosity of the volunteers decreased significantly by the Kampoh prescription. Plasma viscosity showed no significant changes. The malondialdehyde (MDA) production in platelets induced by *N*-ethylmaleimide (NEM) decreased significantly after oral administration of the Kampoh prescription. However, platelet aggregation induced by external reagents (adenosine-5′-diphosphate, epinephrine, and collagen) showed no significant changes, even after Tôki-syakuyaku-san administration. The results indicate that Tôki-syakuyaku-san has the potential to improve "oketsu" syndrome by reducing blood viscosity and suppressing thromboxane synthesis in platelets.

Key words Tôki-syakuyaku-san, blood viscosity, platelet function, malondialdehyde, clinical investigation, Angelicae Radix

Abbreviations ADP, adenosine-5′-diphosphate ; MDA, malondialdehyde ; NEM, *N*-ethylmaleimide ; PPP, platelet poor plasma ; PRP, platelet rich plasma ; Keisi-bukuryô-gan (Gui-Zhi-Fu-Ling-Wan), 桂枝茯苓丸 ; Tôki-syakuyaku-san (Dang-Gui-Shao-Yao-San), 当帰芍薬散

Introduction

In previous papers,[1,2] we reported that the value of blood viscosity in a patient with "oketsu" syndrome is significantly elevated when compared with normal subjects, and also that thromboxane synthetic pathways are significantly accelerated in the syndrome.

Tôki-syakuyaku-san (Dang-Gui-Shao-Yao-San) is thought to be one of the most important Kampoh prescriptions for improving "oketsu" syndrome.[3,4] The present investigation was undertaken in an attempt to reveal the effect of Tôki-syakuyaku-san on blood viscosity and thromboxane synthesis in platelets in normal subjects.

Materials and Methods

Substances : A granulated preparation of Tôki-syakuyaku-san extract (Tsumura Co., Ltd., Tokyo, Lot No. 5014241) was used for this study. The constitution of this prescription is listed in Table I.

Protocol of medication : Twelve healthy volunteers (6 males and 6 females, aged 18—23) made up of students of this college were investigated.

*〒930-01 富山市杉谷 2630
富山医科薬科大学附属病院和漢診療部 鳥居塚和生
2630 Sugitani, Toyama 930-01, Japan

Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 4, 20－25, 1987

Table I Constitution of Tôki-syakuyaku-san extract.

Angelicae Radix	3.0	Paeoniae Radix	4.0
Cnidii Rhizoma	3.0	Hoelen	4.0
Alismatis Rhizoma	4.0	Atractylodis Rhizoma	4.0
			gram

7.5 g of granulated preparation of Tôki-syakuyaku-san contains 3 g of water extract of the above constitution.

By using Terasawa's diagnostic criteria of "oketsu" syndrome,[1] their "oketsu" scores were estimated to be under 20. For one week, they took 7.5 g per day of Tôki-syakuyaku-san extract orally. During the test period, the subjects ate a regular diet and were prohibited from taking alcoholic beverages and drugs which might affect platelet functions. Blood samples were obtained at one week before administration of the testing drug in order to estimate the effects of regular diet on the parameters.

Reagents : Platelet aggregation agents adopted in this study were collagen (Collagen reagent "HORM" from Hormon Chemie Co., Ltd., Munich, West Germany), adenosine-5′-diphosphate (ADP, from Sigma Co., Ltd., St. Louis, MO., USA) and epinephrin (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan). Special grade *N*-ethylmaleimide (NEM) and 2-thio-barbituric acid (Nakarai Co., Ltd., Kyoto, Japan) were employed in the determination of malondialdehyde (MDA). For the other reagents, special grade materials were purchased from Wako-Junyaku Co., Ltd., Osaka, Japan.

Blood samples : The volunteers were asked to have a light breakfast before visiting our clinic where blood samples were taken between 8 : 30 a.m. and 10 : 00 a.m. For the determination of whole blood and plasma viscosity, 6 ml were withdrawn from the cuvital vein into a plastic syringe with ethylenediamine-tetra-acetic-acid-2K (1.8 mg/ml). Each sample was divided into two parts, one for measuring whole blood viscosity and hematocrit, and the other to examine plasma viscosity. All samples were examined at least one hour after sampling.

For the examination of platelet aggregation and MDA production, 14 ml of blood was collected into 3.8% (W/V) sodium citrate (9 : 1). After centrifugation at $150\times g$ for 12 minutes at 23℃, the supernatant was obtained : this fluid was called platelet rich plasma (PRP). The precipitate was further centrifuged at $1100\times g$ for 15 minutes at 4℃, yielding another supernatant ; it was labeled platelet poor plasma (PPP). The number of platelets in PRP was determined with an automatic platelet counting apparatus (PL-100, TOA Medical Electronics Co., Ltd., Tokyo).

Measurement of viscosity : Viscosities of whole blood and plasma were measured by a cone-plate rotational viscometer (Bio-rheolizer, Tokyo Keiki Co., Ltd., Tokyo), as described in a previous paper[1]. The measurements were carried out at 37℃ and a cone angle of 1°38′. For calibration of the viscometer, standard oil solution JS 10 (Syowa Oil Co., Ltd., Tokyo, Lot No. 10) was employed. Whole blood viscosity was estimated at high shear rates (384.0 sec^{-1}), and plasma viscosity was measured under similar conditions. The values of viscosity were calculated by averaging three estimations.

The values of whole blood viscoisity (apparent viscosity) were corrected to a standard hematocrit value of 45% by using the following equation[1] :

$$\log_{10}\eta_{45}=\log_{10}\eta+0.0113(45-\mathrm{Ht})$$

η : apparent viscosity (cp)

Ht : hematocrit (%)

Determination of platelet aggregation : The platelet count of PRP was adjusted to 3×10^5per μl by adding autologous PPP. The aliquot of PRP (200 μl) was placed in a cuvette in an automatic platelet aggregometer, together with one of the platelet aggregation agents, epinephrine (final concentrations : 2.5 μM, 5.0 μM, 10 μM), collagen (final concentrations : 0.5 μg/ml, 1.0 μg/ml, 2.0 μg/ml) and ADP (final concentrations : 1.2 μM, 2.5 μM, 5.0 μM). The measurement of aggrega-

tion was carried out with a NKK Hematracer I (Niko Bio-science Inc., Tokyo), and aggregability of each sample was estimated with the maximal aggregation.

Measurement of MDA production in platelets : MDA production in platelets was determined in order to estimate the activity of enzymes related to prostaglandin production in platelets. The method of measurement was based on those of Smith *et al*.[5] and Stuart *et al*.[6] One hundred μl of the platelet aggregating agent (NEM, 11 mM) was added to 1 ml of PRP and shaken at 37℃ for three minutes. Then an equal volume of 20% (W/V) trichloroacetic acid in 0.6 M hydrochrolic acid solution was added to the mixture and stirred so as to stop the reaction and extract MDA. The amount of MDA (nmol/10^9 platelets) produced in platelets was determined from the calibration curve prepared with known amounts of MDA. The details of this process were described in the previous paper.[2]

Results

Changes in whole blood viscosity and plasma viscosity

The changes in whole blood viscosity and plasma viscosity following oral administration of Tôki-syakuyaku-san were shown in Figs. 1 and 2, respectively. The values of whole blood viscosity decreased significantly ($p<0.05$) after Tôki - syakuyaku - san administration. Meanwhile, the values of plasma viscosity did not change significantly, although they showed some tendency to decrease.

Changes in MDA production in platelets

Fig. 3 shows the changes in MDA production after oral administration of Tôki-syakuyaku-san

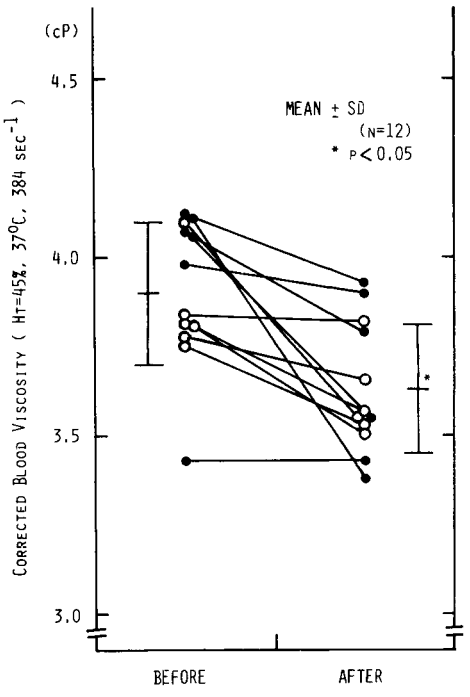

Fig. 1 Effects of Tôki-syakuyaku-san extract on whole blood viscosity in normal subjects.
● : male, ○ : female

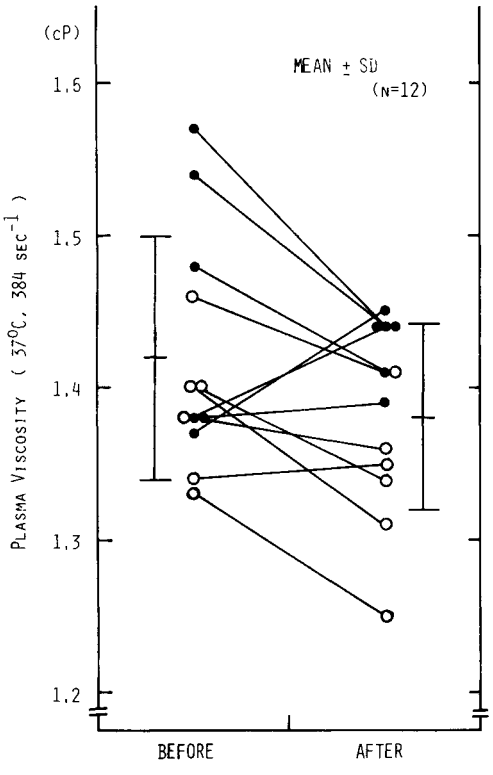

Fig. 2 Effects of Tôki-syakuyaku-san extract on plasma viscosity in normal subjects.
● : male, ○ : female

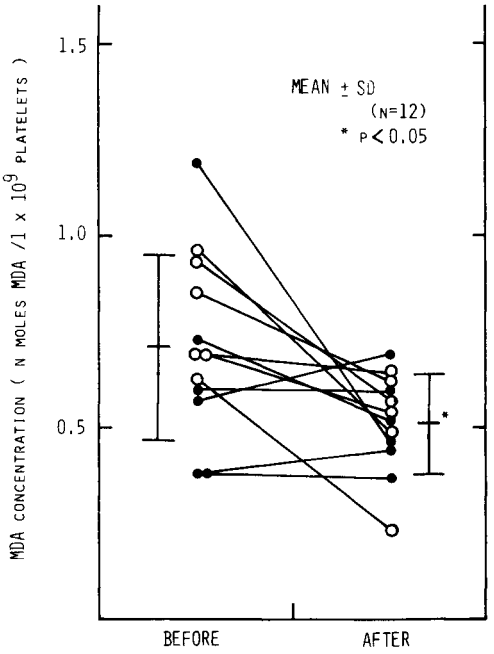

Fig. 3 Effects of Tôki-syakuyaku-san extract on malondialdehyde production in PRP in normal subjects.
● : male, ○ : female

for one week. The results indicate that MDA production in platelets was significantly ($p<0.05$) suppressed by Tôki-syakuyaku-san administration.

Changes in platelet aggregability

Table II shows the changes in platelet aggregability after Tôki-syakuyaku-san administration. Although MDA production in platelet was supressed, significant changes in platelets aggregability induced by external reagents were not observed.

Changes in platelet counts and hematocrit values

No significant changes were observed either in platelet counts and hematocrit values after oral administration of Tôki-syakuyaku-san. In fact, the values of these two parameters remained within normal limits.

Comparing male and female groups

Concerning the initial values of above parameters, there were no significant differences between male and female groups. However, as shown in Table III, some differences were observed in changes of plasma viscosity and MDA production after Tôki-syakuyaku-san administration. No significant differences were demonstrated in platelet aggregability, platelet counts or hematocrit values.

Changes in the parameters during control period

Prior to administration of testing drug, control study to the same subjects was performed to estimate the effects of regular diet on the above parameters. No significant changes in those parameters were found in a one week trial.

Effects of Tôki-syakuyaku-san on overall physical conditions

There were no recognisible subjective complaints such as anorexia, general malaise, diar-

Table II Effect of Tôki-syakuyaku-san extract on the platelet aggregation in normal subjects.

Reagents	Concentration (Final)	Before	After
Epinephrine	2.5 μM	64.0±26.2%	57.5±29.7%
	5.0 μM	73.8±21.6	66.8±21.1
	10.0 μM	72.9±16.4	73.7±12.4
Collagen	0.5 μg/ml	61.9±30.0	57.1±24.2
	1.0 μg/ml	69.8±17.9	74.8± 6.9
	2.0 μg/ml	80.4± 9.9	81.0± 3.9
ADP	1.2 μM	28.1±24.5	26.3±24.8
	2.5 μM	59.6±19.8	54.4±24.1
	5.0 μM	74.2±14.8	66.6±18.4

The values are expressed as mean and standard deviation.

Table III Comparison of effect of Tôki-syakuyaku-san extract in male and female groups.

	group	n	before	after	p value[a]
Blood viscosity[b]	male+female	12	3.91±0.21	3.64±0.18	$p<0.01$
	male	6	3.96±0.27	3.66±0.24	$p<0.05$
	female	6	3.85±0.13	3.61±0.12	$p<0.05$
Plasma viscosity[c]	male+female	12	1.42±0.08	1.38±0.06	N.S.[e]
	male	6	1.45±0.09	1.43±0.02	N.S.
	female	6	1.39±0.05	1.34±0.05	$p<0.05$
MDA production[d]	male+female	12	0.72±0.24	0.51±0.13	$p<0.05$
	male	6	0.64±0.30	0.52±0.12	N.S.
	female	6	0.79±0.14	0.51±0.15	$p<0.01$

The values are expressed as mean and standard deviation.
a) calculated with a paired sample t-test.
b) centi poise, corrected to standard hematocrit of 45%, measured at 384 sec^{-1}.
c) centi poise, measured at 384 sec^{-1}.
d) n moles MDA per 1×10^9 platelets.
e) not significant.

rhea or headache during the experimental period.

Discussion

Tôki-syakuyaku-san is first described in the textbooks "Shan Han Lun"[3] and "Chin Kuei Yao Lueh"[4] (200 A.D.), and this is one of the most important Kampoh prescriptions for "oketsu" syndrome. As listed in Table I, this prescription contains Angelicae Radix, Cnidii Rhizoma and Paeoniae Radix which are thought to improve "oketsu" syndrome.

In previous reports, the authors revealed that the syndrome was closely related to hyperviscosity of blood[1] and accelerated MDA production in platelets.[2] The results of the present investigation indicate that Tôki-syakuyaku-san has the effect of reducing the whole blood viscosity in healthy volunteers, as shown in Figs. 1 and 2. With respect to plasma viscosity, although significant differences were not found, the values tend to decrease.

The results obtained in this study are essentially the same as those of our preceding report using Keisi-bukuryô-gan, which is also thought to be one of the important prescriptions for "oketsu" syndrome.[2] In daily practice in Kampoh medicine, however, Tôki-syakuyaku-san has an indication only for a delicate constitution with "oketsu" syndrome, while Keisi-bukuryô-gan has an indication for a constitution of stenia with the syndrome. In another report,[7] we reported that *Angelica acutiloba* has two types of effect on peripheral circulation, and we discussed that it depends on constitutional factors of the subjects. To elucidate on this constitutional problem, further investigations on patients with an indication for these prescriptions are needed.

As shown in Fig. 3, the present data also indicate that MDA production of platelets is suppressed by oral administration of Tôki-syakuyaku-san in healthy volunteers. In a previous report,[8] the authors found that water extracts of Tôki-syakuyaku-san inhibited platelet aggregation *in vitro*. The results obtained in the present study support this data *in vivo*, although the platelet aggregation induced by external platelet reagents remained within normal limits. Concerning the discrepancy between supressed MDA production thought to be reflected by suppressed thromboxane synthesis in platelets,[5,6,9] and platelet aggregation induced by external reagents, it can be considered that platelet aggregation is caused not only by thromboxane alone but by many other factors as well. This result is essentially the same as that of Keisi-bukuryô-gan ad-

ministration in healthy volunteers.[2)]

There remains the possibility that the effects of this drug is due to binding agents such as starch or sucrose. However, starch and sucrose are usually contained in a regular diet, and the results obtained indicate that a regular diet did not have an affect on those parameters mentioned above. So that, the effects observed in this study are thought to be mainly brought by Tôki-syakuyaku-san itself.

The effects expected of Tôki-syakuyaku-san in traditional Kampoh medicine are many, such as anti-"oketsu" effect, anti-spasmodic effect, analgesic, immunomodulator, endocrine-regulator, especially the sexual hormone system, and anti-inflammatory effects.[3,4)] And this prescription has indications for women's disease such as amenorrhea, climacteric disorders or miscarriage. The results obtained in this study indicate that this prescription is more effective in the female rather than male group, which are thought to be closely related to the properties of this drug. However, the reason why this discrepancy occurred in each group still remains unclear. So that, further investigations on this problem are called for.

The present data only revealed a portion of the effects of this prescription. However, it was suggested that Tôki-syakuyaku-san has the potential to improve peripheral micro-circulation by reducing blood viscosity and thromboxane synthesis in platelets.

Acknowledgement

This investigation was supported by a Grant-in-Aid for scientific research of Kampoh medicine from Tsumura Juntendo Co., Ltd., Tokyo, Japan.

和文抄録

当帰芍薬散の血液粘度，血小板機能に対する効果について検討した。12名の健常人に対し当帰芍薬散エキス製剤を7.5 g/day で 1 週間投与し，全血粘度，血漿粘度，ヘマトクリット値，血小板数，血小板凝集能，malondialdehyde (MDA) 産生量について投与前後の値を比較した。全血粘度，血漿粘度は投与後では低下し，全血粘度ではその差は有意であった。血小板凝集能はコラーゲン，エピネフリン，ADP を凝集惹起物質として用い検討したが，有意な変化は認められなかった。一方 MDA 産生能は有意に抑制された。ヘマトクリット値，血小板数に変化は認められず，いずれも正常値内であった。これらの成績は，当帰芍薬散は血液流動性や血小板内 cyclo-oxygenase 系に作用することを示唆している。

References

1) Terasawa, K., Toriizuka, K., Tosa, H., Ueno, M., Hayashi, T. and Shimizu, M. : Rheological studies on "oketsu" syndrome I. The blood viscosity and diagnostic criteria. *J. Med. Pharm. Soc.WAKAN-YAKU* **3**, 98-104, 1986

2) Terasawa, K., Toriizuka, K., Bandou, M., Imadaya, A., and Tosa, H. : Effects of medicinal plants on the metabolism of platelet arachidonic acid—Studies on "oketsu" syndrome, platelet aggregation and changes in malondialdehyde values. *J. Med. Pharm. Soc. WAKAN-YAKU* **2**, 310-316, 1985

3) Chang Chung-chin : "Shan Hang Lun" (Eds. by Hong-yen Hsu and W.G. Peacher), Oriental Healing Arts Institute, Los Angels, 1981

4) Chang Chun-chin : "Chin Kuei Yao Lueh" (Eds. by Hong-yen Hsu and Su-yen Wang), Oriental Healing Arts Institute, Los Angels, 1983

5) Smith, J.B., Ingerman, C.M. and Silver, M.J. : Malondialdehyde as an indicator of prostaglandin production by human platelets. *J. Lab. Clin. Med.* **88**, 167-172, 1976

6) Stuart, M., Murphy, S. and Oski, F.A. : A simple nonradioisotope technic for the determination of platelet life span. *New Engl. J. Med.* **292**, 1310-1313, 1975

7) Terasawa, K., Imadaya, A., Tosa, H., Mitsuma, T., Toriizuka, K., Takeda, K., Mikage, M., Hattori, M. and Namba, T. : Chemical and clinical evaluation of crude drug derived from *Angelica acutiloba* and *A. sinnensis*. *FITOTERAPIA* **LVI**, 201-208, 1985

8) Terasawa, K., Kimura, M., Sakuragawa, N., Uchiyama, Y., Toriizuka, K., Ueno, M. and Horikoshi, I. : Effects of anti-"oketsu" drugs on blood coagulation and fibrinolysis. *Yakugaku Zasshi* **103**, 313-318, 1983

9) Okuma, M., Takayama, H. and Uchino, H. : A simple method for estimation of lipoxigenase and cyclo-oxygenase pathways in human platelets—The use of thiobarbituric acid reaction. *Thrombos. Haemostas.* **42**, 245, 1979