

Effects of Keisi-bukuryô-gan on the microcirculation of  
bulbar conjunctiva in normal subjects

Takashi ITOH, Katsutoshi TERASAWA,\* Yumi MORIMOTO, Hiroyori TOSA and Yukitaka HIYAMA

*Department of Japanese Oriental Medicine, Toyama Medical and Pharmaceutical University**(Received October 27, 1988. Accepted November 28, 1988.)*

## Abstract

In order to elucidate the effects of Keisi-bukuryô-gan on the microcirculation of bulbar conjunctiva, 35 healthy volunteers (18–57 y.o.), 6 of whom served as control, were evaluated in this study. According to Terasawa's diagnostic criteria of the "oketsu" syndrome, the volunteers were classified into three groups. By using a video-microscope system, the blood flow rate and internal diameter of the microvessels were measured by regenerated pictures on a TV monitor obtained both before and after Keisi-bukuryô-gan administration. Changes in blood pressure and pulse rate were also measured at the same time. The results indicated that both the blood flow rate and internal diameter of microvessels increased significantly following the oral administration of this formula, but the blood pressure and pulse rate were not changed significantly. In the two groups of the "oketsu" state, there was significant increase of blood flow volume after the administration of Keisi-bukuryô-gan, but it was not significant compared with the changes in the non-"oketsu" group.

**Key words** Keisi-bukuryô-gan, microcirculation, bulbar conjunctiva, video-microscope system, normal subjects.

**Abbreviations** FVe, blood flow velocity; FVo, blood flow volume; ID, internal diameter of vessel; Keisi-bukuryô-gan (Gui-Zhi-Fu-Ling-Wan), 桂枝茯苓丸; "oketsu" (Yu-Xue), 瘀血.

## Introduction

In the previous paper,<sup>1)</sup> we reported that the empirical concept of the "oketsu" syndrome is closely correlated with abnormalities of the microcirculation, such as intravascular erythrocyte agglutination with sludging phenomenon, stasis and/or narrowing of microvessels. It was also observed that the blood flow rate was sometimes remarkably reduced in this syndrome.

Keisi-bukuryô-gan (Gui-Zhi-Fu-Ling-Wan) is one of the most popular Kampo formulas for treating the "oketsu" syndrome. The pharmacological effects of this formula have been reported as follows: improving Raynaud phenomenon,<sup>2)</sup> lowering blood viscosity,<sup>3)</sup> and decreasing

thromboxane synthesis of platelets,<sup>4)</sup> all suggesting that this formula possesses the potential to improve the microcirculation.

Recently, Hayashi *et al.*<sup>5)</sup> have reported on the pharmacological effects of this formula on the microcirculation of bulbar conjunctiva in patients with the "oketsu" syndrome. However, theirs was not a controlled study. The present study was undertaken in an attempt to determine the effects of Keisi-bukuryô-gan on the microcirculation of bulbar conjunctiva in both test and control groups by using a video-microscope system.

## Subjects and Methods

**Substances**: Keisi-bukuryô-gan prepared by the hospital pharmacy of the Toyama Medical

\*〒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 寺澤捷年  
2630 Sugitani, Toyama 930-01, Japan.

and Pharmaceutical University was used in this study. One six-gram pill consists of 3.0 grams of honey and the following five medicinal plants : Cinnamomi Cortex 0.6 g, Polia 0.6 g, Moutan Cortex 0.6 g, Persicae Semen 0.6 g and Paeoniae Radix 0.6 g.

*Medication protocol* : Thirty-five healthy volunteers (males) consisting of students and staff of this university were investigated. They were divided into two groups : 29 of them made up the test group, and 6 of them served as the control group. The age of the two groups were  $25.1 \pm 5.3$  (mean  $\pm$  S.D.) and  $23.5 \pm 6.2$  years, respectively. Consent was obtained from the individuals. After overnight fasting, the volunteers visited our department at 9 : 00 a.m.. By using Terasawa's diagnostic criteria of the "oketsu" syndrome,<sup>61</sup> their "oketsu" scores were estimated. After 30 minutes of bed rest, blood pressure and pulse rate were measured, and then their microcirculation

of bulbar conjunctiva was evaluated by using a video-microscope system.<sup>11</sup> Following the video-recording, they were administered 6.0 grams of Keisi-bukuryō-gan with 200 ml of hot water (37°C) in the test group, or 200 ml of hot water only (37°C) in the control group. After one hour of bed rest, blood pressure and pulse rate were checked again, and then their microcirculation of the bulbar conjunctiva was re-evaluated.

*Measurement of blood flow rate and internal diameter of microvessels* : The details of the video-microscope system were given in the previous paper.<sup>11</sup> For measuring of the blood flow rate and internal diameter of microvessels, a video timer (VTG-33, Hoei Co., Ltd., Tokyo) and video measuring gauge (IV-560, Hoei Co.) were used. The measurement procedure of the blood flow rate is illustrated and explained in Fig. 1.

*Statistical analysis* : For analysis of the changes in parameters in the control group vs.

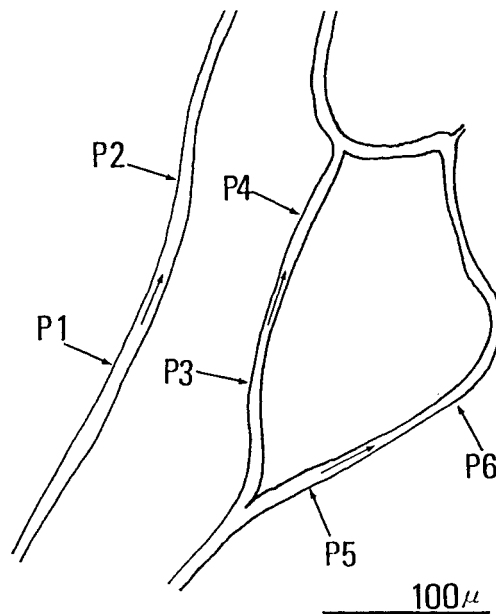


Fig. 1 The principle of measuring of the blood flow rate in microvessels : In each subject, three different parts of vessels (about 8–10  $\mu$ m in diameter) are chosen, and the distances of P1–P2, P3–P4 and P5–P6 are measured by a measuring gauge connected to a TV monitor. Then the passage of time of a lump of erythrocytes or a white blood cell between P1–P2, P3–P4 and P5–P6 is calculated by a video-timer. In practice, the regenerated pictures are enhanced by a video enhancer (RE-11, Hoei Co., Ltd.) which makes the detection of a lump of cells in the vessel much easier. The display speed of the regenerated pictures is also controlled by a remote control unit (RM-70, Victor Co., Ltd.) in order to facilitate the reading time (msec) on a TV monitor.

the testing group, or before vs. after in the three groups of the "oketsu" state, the Student's *t*-test was used.

### Results

#### *Changes in the blood flow rate and internal diameter of microvessels*

The results are listed in Tables I—III. As shown in Table I, the blood flow rate increased about 13% by Keisi-bukuryō-gan administration, which was significant when compared with controls. The internal diameter (ID) of microvessels also increased significantly following the administration of this formula (Table II). By using the following equation, the blood flow volume (FVo)

Table I Changes in blood flow rate after oral administration of Keisi-bukuryō-gan.

	Before	After	Difference
Test group	3.04±0.64	3.44±0.71	0.40±0.4
Control group	3.42±0.59	3.30±0.59	-0.12±0.4 (mm/sec)

Note : mean±S.D.,  $p < 0.001$ , the difference between active and control.

Table II Changes in internal diameter of microvessels after oral administration of Keisi-bukuryō-gan.

	Before	After	Difference
Test group	9.3±3.2	9.7±3.7	0.39±1.6
Control group	9.3±1.9	8.8±1.5	-0.44±1.0

Note : mean+S.D.  $\mu\text{m}$ ,  $p < 0.05$ , the difference between active and control.

Table III Changes in blood flow volume of microvessels after oral administration of Keisi-bukuryō-gan.

	Before	After	Difference
Test group	3.6±3.2	4.6±5.5	3.97±0.01
Control group	3.6±1.7	3.1±1.2	-1.96±3.5

Note : mean±S.D.,  $\text{X}10^{-3} \text{ mm}^3/\text{sec}$ ,  $p < 0.01$ , the difference between active and control.

Table IV Changes in systolic/diastolic pressure and pulse rate after oral administration of Keisi-bukuryō-gan.

	Before	After	Difference
Systolic pressure			
Test group	117.9± 9.7	118.0±9.6	0.10±0.5
Control group	117.9± 9.8	111.0±6.4	-0.67±7.0 (mmHg)
Diastolic pressure			
Test group	68.8±10.0	70.0±8.7	1.24±7.7
Control group	59.7± 4.3	59.5±4.2	-0.17±5.7 (mmHg)
Pulse rate			
Test group	66.9± 9.0	63.9±9.1	-3.00±5.7
Control group	64.7± 8.6	61.7±9.7	-3.00±3.5 (per min)

Note : mean±S.D., not significant : active vs. control.

Table V Changes in blood flow volume in the three groups of the "oketsu" state after the oral administration of Keisi-bukuryō-gan.

	Before	After	<i>p</i> value
Non-"oketsu" (n=10×3)	4.09±4.33	5.49±8.24	NS
Mild "oketsu" (n=14×3)	3.40±2.04	4.15±2.57	<0.05
Severe "oketsu" (n=5×3)	3.24±3.24	4.10±3.99	<0.05

Note: mean±S.D., × 10<sup>-3</sup> mm<sup>3</sup>/sec.

n: number of subjects × three sections of evaluated vessels in each subject.

*p* value: before vs. after.

in Table III was obtained:

$$FV_o \text{ (mm}^3\text{/sec)} = (1/2 \text{ ID})^2 \times \pi \times FVe$$

The results indicate that FV<sub>o</sub> increased about 28% after oral administration of the testing drug.

#### Changes in the blood pressure and pulse rate

The systolic/diastolic blood pressure and pulse rate are listed in Table IV. There were no significant changes in these parameters when they were compared with those of the controls.

Correlation between the "oketsu" state and blood flow volume: As shown in Table V, there was significant increase of blood flow volume in the group of the "oketsu" state, but it was not significant statistically in comparison with the changes in the non-"oketsu" group.

### Discussion

Recently, Hayashi *et al.*<sup>5)</sup> reported that Keisi-bukuryō-gan has the effect of increasing the blood flow rate and internal diameter of the microvessels of bulbar conjunctiva in patients with the "oketsu" syndrome. However, their report was not based on a controlled study. Therefore, there remains some uncertainty as to whether this formula actually increases these two parameters in compared with controls.

In this study, 6 volunteers served as control. As shown in Tables I–V, there were no significant differences between the two groups regarding the values of the various parameters before administration of the test drug and/or hot water. Under such well matched conditions, the blood

flow rate increased significantly after administration of the test drug. The changes in the internal diameter of the microvessels also increased significantly compared with control.

Concerning the pharmacological effects of the medicinal plants contained in Keisi-bukuryō-gan on the cardiovascular systems, Wan *et al.*<sup>7)</sup> revealed that the methanol extracts of both Persicae Semen and Moutan Cortex relax the isolated aortic strips precontracted with noradrenaline. Harada *et al.*<sup>8)</sup> reported that cinnamaldehyde derived from Cinnamomi Cortex increases the peripheral blood flow, and decreases both blood pressure and heart rate in the anesthetized dog. Paeoniflorin, a main component of Paeoniae Radix, decreases blood pressure and heart rate in the anesthetized guinea-pig.<sup>9)</sup> Taken together, these studies suggest that Keisi-bukuryō-gan may have some effects on peripheral blood flow, heart rate and blood pressure. However, the results obtained in the present study indicate that Keisi-bukuryō-gan does not effectively influence the heart rate and blood pressure, but it does have an effect on blood flow volume in normal subjects.

In our previous study,<sup>3)</sup> we reported that this formula decreases both whole blood viscosity and plasma viscosity in normal subjects. Therefore, it is assumed that the main factors influencing the increased blood flow rate and internal diameter observed in this study are due to both vasodilatation and lowered blood viscosity brought about by the Keisi-bukuryō-gan administration. However, it is also well known that lowered blood vis-

cosity may increase cardiac output.<sup>10)</sup> Therefore, further investigations concerning the changes in cardiac output after administration of this formula are called for.

In any case, through this investigation it has become apparent that Keisi-bukuryō-gan has the effect of increasing the blood flow volume in the microcirculation, at least as far as the microvessels of bulbar conjunctiva of normal subjects are concerned.

### Acknowledgement

We express our gratitude to Mr. A. Gerz for his critical reading of this manuscript. This work was supported in part by a research fund from Tochimoto Tenkaido Co., Ltd., Osaka, Japan.

### 和文抄録

桂枝茯苓丸のヒト眼球結膜微小循環に対する効果を明らかにする目的で、35人の男性、正常人被検者(年齢18-57才)について検討した。35人中6人を対照群とした比較試験である。また寺澤らの瘀血診断基準により被検薬投与群29人を3群に分けた解析も行った。眼球結膜微小血管の観察はビデオ顕微鏡システムを用いて行い、桂枝茯苓丸投与前と投与後の血管内径と流速の変化を再生録画により計測した。血圧と脈拍についても検討した。その結果、桂枝茯苓丸は血管内径と流速を共に有意に増加させることが明らかになった。しかし血圧、脈拍には影響を与えなかった。瘀血診断基準により分類した瘀血群においては桂枝茯苓丸投与により血管内径、流速ともに明らかな増加がみられたが、非瘀血群の変化率を検定の対照とすると推計学的な有意差は得られなかった。

### References

- 1) Terasawa, K., Itoh, T., Morimoto, Y., Hiyama, Y. and Tosa, H. : The characteristics of the microcirculation of bulbar conjunctiva in "oketsu" syndrome. *J. Med. Pharm. Soc. WAKAN-YAKU* **5**, 200-205, 1988.
- 2) Terasawa, K., Matsuda, H., Imadaya, A., Tosa, H., Mitsuma, T., Toriizuka, K. and Homma, S. : A study on clinical effects of Kuei-chih-fu-ling-wan prepared in the hospital pharmacy. *Jap. J. Orient. Med.* **35**, 131-136, 1984.
- 3) Tosa, H., Toriizuka, K. and Terasawa, K. : The effect of Keisi-bukuryō-gan on blood viscosity, platelet functions and blood coagulation in normal subjects. *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 172-179, 1987.
- 4) Terasawa, K., Toriizuka, K., Bandou, M., Imadaya, A., and Tosa, H. : Effects of medicinal plants on the metabolism of platelet arachidonic acid. *J. Med. Pharm. Soc. WAKAN-YAKU* **2**, 310-316, 1985.
- 5) Hayashi, T., Tsushima, N., Sakakura, M., Gama, M., Koishi, M., Tamai, T., Fujiyoshi, Y., and Yasunaga, K. : Changes in microcirculation of human bulbar conjunctiva and rheology of blood after administration of Keisi-bukuryō-gan. *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 358-359, 1987.
- 6) Terasawa, K., Shinoda, H., Imadaya, A., Tosa, H., Bandou, M. and Satoh, N. : The presentation of diagnostic criteria for "oketsu" syndrome. *Jap. J. Orient. Med.* **34**, 1-17, 1983.
- 7) Wan, S., Yano, S., Watanabe, K., Tanaka, O. and Shoji, J. : Pharmacological effects of anti-"oketsu" drugs. *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 274-275, 1987.
- 8) Harada, M. and Yano, S. : Pharmacological studies on Chinese Cinnamon. II. Effects of cinnamaldehyde on the cardiovascular and digestive systems. *Chem. Pharm. Bull.* **23**, 941-947, 1975.
- 9) Takagi, K. and Harada, M. : Pharmacological studies on Paeoniae Radix (III). Effects of paeoniflorin on cardiovascular and respiratory systems, and isolated organs. *Yakugaku Zasshi* **89**, 893-898, 1969.
- 10) Fowler, N.O. and Holmes, J.C. : Blood viscosity and cardiac output in acute experimental anemia. *J. Appl. Physiol.* **39**, 453-456, 1975.