

Effects of Oren-gedoku-to on the microcirculation of bulbar conjunctiva in normal subjects

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Abstract

In order to elucidate the effect of Oren-gedoku-to on the microcirculation of bulbar conjunctiva, 10 healthy volunteers (male, 20-42 y.o.) were evaluated in this study. By using a video-microscope system, blood flow velocity and the internal diameter of microvessels were measured by re-generated pictures on a TV monitor. Keishi-bukuryo-gan, which affects the microcirculation reported previously, was also evaluated in order to determine the differences in the mode of action between the two drugs. The results indicated that Oren-gedoku-to decreased blood flow in superficial vessels and increased that in deep layers. In contrast, Keishi-bukuryo-gan increased blood flow volume in both the superficial and deep layers. The differences observed in this study were partly due to the anatomical characteristics of bulbar conjunctiva: The blood supply of the microvessels of the superficial layer comes under the influence of both the external and internal carotid arteries, vessels of the deep layers mainly receive their blood supply by way of the optic artery originating from the internal carotid artery.

Key words Keishi-bukuryo-gan (Keishi-bukuryo-gan), Oren-gedoku-to (Oren-gedoku-to), microcirculation, Coptidis Rhizoma, Scutellariae Radix, Gardeniae Fructus, Phellodendri Cortex.

Abbreviations FVe, blood velocity; FVo, blood flow volume; ID, internal diameter; Keishi-bukuryo-gan (Gui-Zhi-Fu-Ling-Wan), 桂枝茯苓丸; Oren-gedoku-to (Huang-Lian-Jie-Du-Tang), 黄连解毒湯.

Introduction

Recently several papers have reported that Oren-gedoku-to improves the symptoms associated with cerebro-vascular type of dementia.^{1,2)} Kogure²⁾ revealed that this prescription increased the blood flow in ischemic brain tissue induced by the artificially occluded internal carotid artery.

However, there has been no report about the effects of Oren-gedoku-to on microcirculation. In the previous paper,³⁾ we reported how Keishi-bukuryo-gan influenced the microcirculation in bulbar conjunctiva. The present study was undertaken in an attempt to determine the effects of Oren-gedoku-to on the microcirculation of bulbar conjunctiva by using a video-microscope

system, and also to elucidate the different mode of action between Keishi-bukuryo-gan and this prescription.

Subjects and Methods

Substances:

1) Oren-gedoku-to (Table I) was prepared as a 200 ml decoction (40°C) just before each experiment. All of it was administered to each subject.

2) Keishi-bukuryo-gan prepared by the hospital pharmacy of the Toyama Medical and Pharmaceutical University was used in this study: One two-gram pill consisted of 1.0 g of honey and the following five medical plants: Cinnamomi Cortex 0.2 g, Poria 0.2 g, Moutan Cortex 0.2 g,

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Table I Medical plants of Oren-gedoku-to.

Name of medical plants	Weight (gram)	Origin
Coptidis Rhizoma	3.0	Tamba area
Scutellariae Radix	2.0	China
Gardeniae Fructus	2.0	San'in area
Phellodendri Cortex	2.0	Korea

Oren-gedoku-to was administered as a 200 ml decoction (40°C), gently boiling the above crude drugs in 250 ml of water for about 40 minutes.

Persicae Semen 0.2 g and Paeoniae Radix 0.2 g. Three pills (6.0 g) of Keishi-bukuryo-gan was administered to each subject with 200 ml of hot water (40°C).

3) As a non-active control, 200 ml of hot water (40°C) was used.

Medication protocol : Ten healthy male subjects aged 20–42 y.o. (average 30.0 ± 6.9 S.D.) participated in the study and informed consent was obtained. The following three agents, Oren-gedoku-to, Keishi-bukuryo-gan and hot water were given to each individual subject on separate days at an interval of more than one week. After overnight fasting, the subjects visited our department at 8:00 a.m. By using Terasawa's diagnostic criteria for the "oketsu" syndrome,⁴⁾ their "oketsu" scores were estimated. After 30 minutes of bed rest, their microcirculation of bulbar conjunctiva was evaluated by using a video-microscope system.⁵⁾ Then they were administered the medicines or hot water. After one hour of bed rest, their microcirculation was re-evaluated. Blood pressure, pulse rate, blood viscosity (whole blood and plasma viscosity), blood count and serum biochemistry (WBC, RBC, Hb, Ht, ChE, GOT, CPK, amylase, blood sugar, T. Chol., Na, K, Cl, Ca, P, fibrinogen) were evalu-

ated both before and after administration of the test drugs or hot water.

Measurement of internal diameter (ID) and blood flow velocity (FVe) of the microvessels : The details of the video-microscope system and measurement procedures of ID and FVe were given in the previous papers.^{5,6)} For the measurement of ID and FVe, a video timer (VTG-33, Hoei Co., Ltd., Tokyo) and video measuring gauge (IV-560, Hoei Co.) were used.

Measurement of capillary network areas on bulbar conjunctiva : The cumulative areas of the capillary network were calculated with a color image analyzer (Olympus CIA102, Tokyo). The still color photos ($\times 5$) obtained with a camera were printed at a size of 25 \times 30 cm, and then the blood vessels in the same regions of bulbar conjunctiva both before and after the medication were analyzed comparatively. In order to obtain suitable photos for this comparative study, 16 still photos were taken in each case both before and after medication. From these, suitable photos were then selected.

Statistical analysis : The significance of the differences in measurement between the control, Oren-gedoku-to and Keishi-bukuryo-gan treated groups was tested by the Student's *t*-test.

Table II The microcirculation of bulbar conjunctiva following the oral administration of Oren-gedoku-to and Keishi-bukuryo-gan.

Agents	Parameters	Time course				
		30	60	90	120	(min)
Keishi-bukuryo-gan (n=9)	Δ ID	5.4 ± 11.0	$21.2 \pm 15.0^{**}$	$15.4 \pm 16.0^*$	15.0 ± 21.3	
	Δ FVe	8.1 ± 33.0	4.3 ± 28.5	5.6 ± 24.5	8.3 ± 30.3	
Oren-gedoku-to (n=9)	Δ ID	-5.7 ± 22.4	$-16.7 \pm 14.5^{**}$	$-32.7 \pm 26.4^*$	$-17.7 \pm 18.0^*$	
	Δ FVe	15.7 ± 31.6	4.6 ± 18.4	$-16.6 \pm 13.9^*$	$-13.7 \pm 15.1^*$	(%)

Note : Mean \pm S.D., $^{**}p < 0.01$, $^*p < 0.05$; before vs. after the administration of each agent.

Preliminary experiments: In order to determine the most suitable time to evaluate the effects of the test drugs, we examined the time courses of the microcirculation of bulbar conjunctiva of the three subjects after the administration of the test drugs (Table II). From these results, we measured the parameters of microvessels at 60 min after the administration of each agent.

Results

Prevalence of "oketsu" scores

The "oketsu" scores of 5 of the 10 subjects ranged between 11 and 17 points (non-"oketsu" group) and the other 5 subjects were from 26 to 31 points (mildly affected "oketsu" group).⁴⁾ The average "oketsu" scores were 21.3 ± 10.1 S.D. points. No subject was estimated to have a severe "oketsu" state (more than 41 points).

Changes in internal diameter (ID) and blood flow velocity (FVe) of the microvessels

The results are listed in Figs. 1–3. In case of hot water administration only (control), there was no change in the ID value (Fig. 1). Follow-

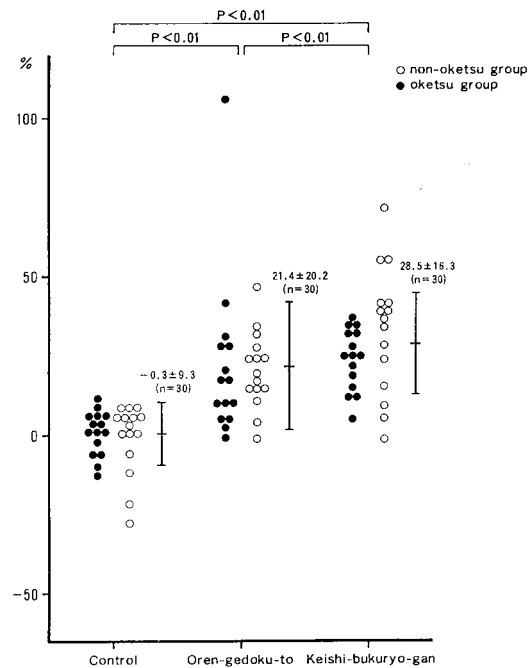


Fig. 2 Changes in Blood Flow Velocity (FVo) on bulbar conjunctiva following the oral administration of Oren-gedoku-to and Keishi-bukuryo-gan.

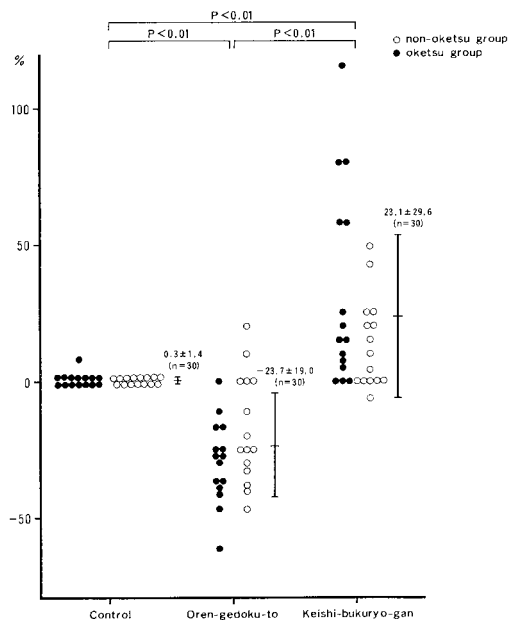


Fig. 1 Changes in Internal Diameter (ID) on bulbar conjunctiva following the oral administration of Oren-gedoku-to and Keishi-bukuryo-gan.

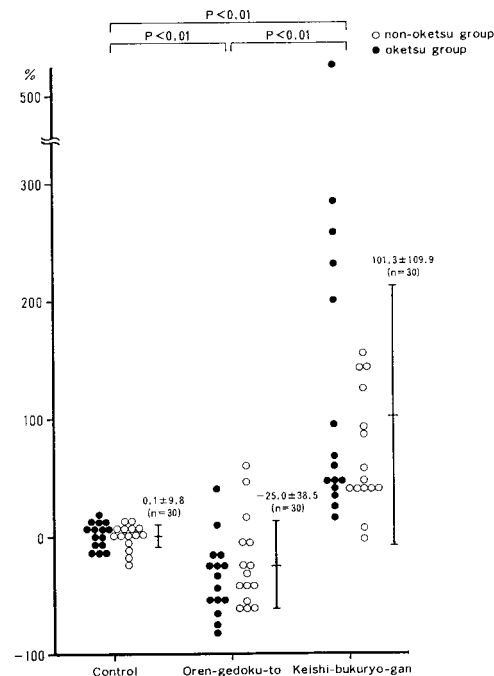


Fig. 3 Changes in Blood Flow Volume (FVo) on bulbar conjunctiva following the oral administration of Oren-gedoku-to and Keishi-bukuryo-gan.

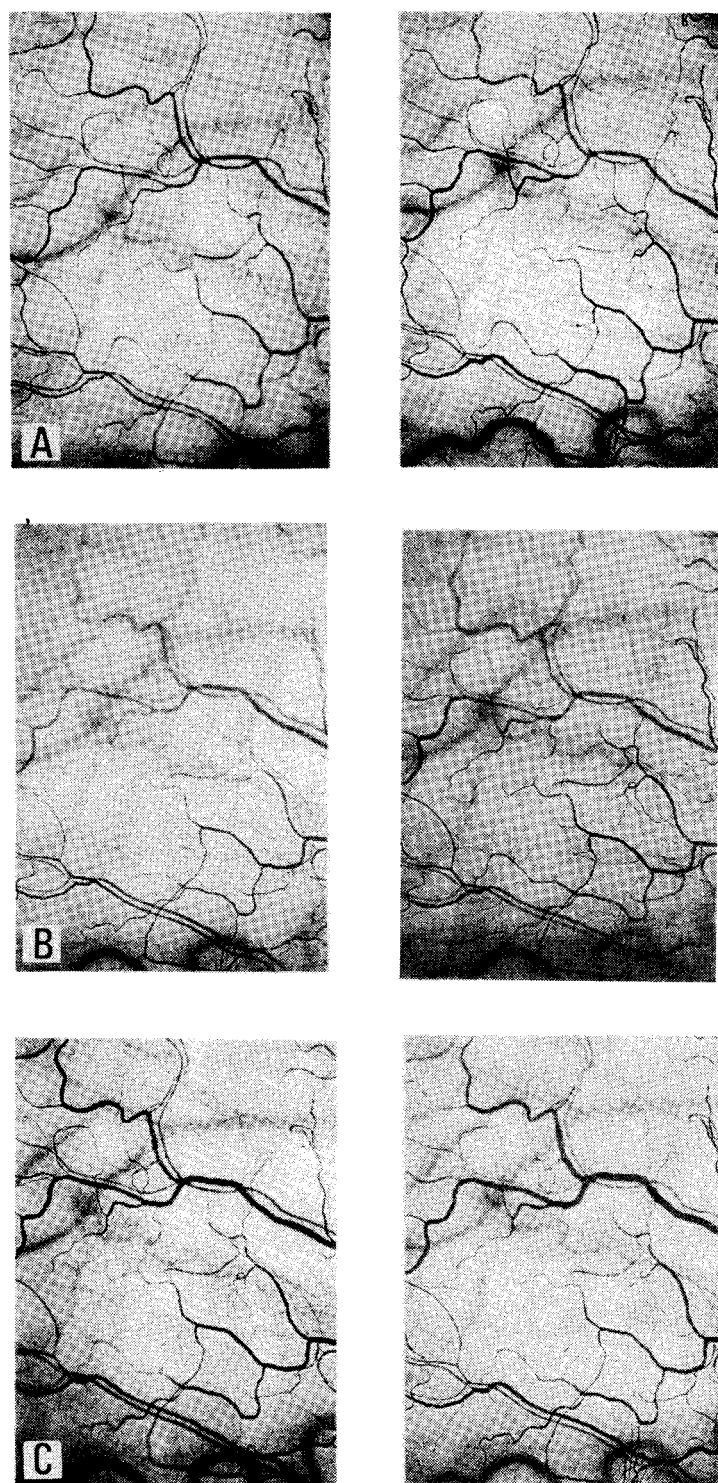


Fig. 4 Changes in capillary network following the administration on hot water (A), Oren-gedoku-to (B), Keishi-bukuryo-gan (C) in the same subject.

Left : before ; Right : after one hour of administration of the test drug.

ing the administration of Keishi-bukuryo-gan, ID increased by 23.1 ± 29.6 S.D.%, but ID decreased 23.7 ± 19.0 S.D.% with Oren-gedoku-to. When the volunteers were divided into non-"oketsu" and "oketsu" groups, the "oketsu" group showed a greater ID rise than the non-"oketsu" group with Keishi-bukuryo-gan, although the difference was not statistically significant. As shown in Fig. 2, following the administration of Keishi-bukuryo-gan and Oren-gedoku-to, FVe increased 28.5 ± 16.3 S.D.% and 21.4 ± 20.2 S.D.%, respectively.

Blood flow volume (FVo) in Fig. 3 was obtained by using the following equation :

$$FVo \text{ (mm}^3\text{/sec)} = \left(\frac{ID}{2}\right)^2 \times \pi \times FVe \text{ (mm/sec)}$$

The results indicated that FVo increased 101.3 ± 109.9 S.D.% following the administration of Keishi-bukuryo-gan, but decreased 25.0 ± 38.5 S.D.% in case of Oren-gedoku-to. Following the administration of Keishi-bukuryo-gan, the change in FVo values in the "oketsu" group was more prominent than in the non-"oketsu" group, although it was not statistically significant.

Changes in capillary network areas in bulbar conjunctiva

Representative photos to illustrate the changes in the capillary network in bulbar con-

Table III Changes in capillary network areas in bulbar conjunctiva following the oral administration of Oren-gedoku-to and Keishi-bukuryo-gan from still photos with a color image analyzer (Olympus CIA102, Tokyo).

Subjects	Control	Oren-gedoku-to	Keishi-bukuryo-gan
No. 1 =	— 9	1548	— 3
No. 2 =	48	1528	34
No. 3 =	— 4	231	1145
No. 4	0.7	111	132
No. 5	—22	38	263
No. 6	—22	316	8
No. 7	8	17	3
No. 8	5	66	9
No. 9	—40	405	110
No. 10	25	49	99 (%)
Mean \pm S.D. (No. 4–10)	-6.5 ± 22.3	$143.1 \pm 153.4^*$	$89.1 \pm 94.1^*$

Note : # Extraordinary high responded cases, these three cases were not involved in this comparative analysis. * $p < 0.05$; active *vs.* control.

Table IV Changes in systolic/diastolic pressure and pulse rate.

	Before	After	Differences
Systolic pressure			
Controls	121.7 ± 15.4	119.3 ± 13.3	-2.4 ± 10.8
Keishi-bukuryo-gan	121.7 ± 15.4	119.7 ± 18.5	-2.0 ± 9.0
Oren-gedoku-to	115.4 ± 13.6	116.1 ± 20.4	0.7 ± 13.3 (mmHg)
Diastolic pressure			
Control	68.2 ± 12.8	66.0 ± 11.5	-2.2 ± 5.9
Keishi-bukuryo-gan	67.6 ± 13.4	67.6 ± 12.1	0.0 ± 4.6
Oren-gedoku-to	66.4 ± 12.9	62.7 ± 13.1	-3.7 ± 5.7 (mmHg)
Pulse rate			
Controls	68.4 ± 10.4	66.3 ± 10.3	-2.1 ± 6.9
Keishi-bukuryo-gan	65.4 ± 10.4	62.7 ± 8.8	$-2.7 \pm 2.8^*$
Oren-gedoku-to	68.9 ± 11.4	62.1 ± 9.0	$-6.8 \pm 6.9^*$ (/min)

Note : mean \pm S.D., * $p < 0.05$, before *vs.* after, not significant ; active *vs.* control.

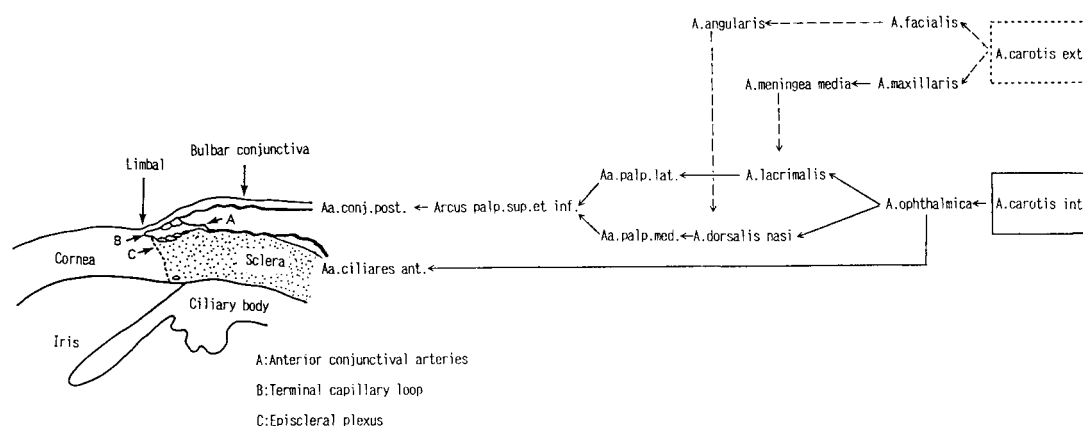


Fig. 5 The schema of bulbar conjunctiva and blood supplying arteries.

junctiva are listed in Figs. 4-A, B and C.

The capillary network areas were calculated from these photos by color image analyzer. As shown in Table III, controls (hot water alone) showed essentially no change or a slight decrease (-6.5 ± 22.3 S.D.%). However, following the administration of Oren-gedoku-to and Keishi-bukuryo-gan, the capillary network areas of bulbar conjunctiva increased 143.1 ± 153.4 S.D.% and 89.1 ± 94.0 S.D.%, respectively, statistically significant in comparison with controls.

Three subjects (No. 1-3) with very extreme responses were not involved in this comparative analysis.

Changes in other parameters

Changes in systolic/diastolic blood pressure and pulse rate are listed in Table IV. Following Oren-gedoku-to and Keishi-bukuryo-gan administration the pulse rate decreased -6.8 ± 6.9 S.D. and -2.7 ± 2.8 S.D. per min ($p < 0.05$; before vs. after), respectively. There were no significant changes in blood pressure and pulse rate when compared with controls.

None of the other parameters such as blood count and blood chemistry were changed significantly.

Discussion

In the previous paper,³⁾ we used a video-microscope system and reported that Keishi-bukuryo-gan possessed the potential to improve

microcirculation in patients with chronic cerebrospinal vascular lesions. In this comparative study, it was revealed that Oren-gedoku-to decreased ID and FVo, and increased FVe and capillary network areas significantly. Keishi-bukuryo-gan on the other hand, increased all of these parameters significantly. These results indicated that the mode of action of the two drugs could be considered to be different.

According to an authoritative textbook of anatomy,⁷⁾ the blood supply of bulbar conjunctiva in humans runs as is described in Fig. 5. This shows that there are two layers of conjunctival vessels, i.e., superficial and deep layers.

The blood supply of the superficial layer is provided by terminal branches of the posterior conjunctival arteries and that of the deep layer comes up the anterior ciliary arteries. Both arteries are derived from the optic artery which originated from the internal carotid artery. However, as illustrated in Fig. 5, the posterior conjunctival arteries partially accept blood by way of the external carotid artery. Therefore, one possibility is that the anatomical difference between the superficial and deep layers of microvessels may be responsible for the different mode of action observed in Keishi-bukuryo-gan and Oren-gedoku-to administration.

The microvessels in bulbar conjunctiva via a TV monitor were seen as a superficial layer because of the easy detection of a lump of erythrocytes or white blood cells enabling the measur-

ing of ID and FVe at high magnification ($\times 175$). On the other hand, in case of still camera photos ($\times 5$), it was possible to observe changes both in the superficial and deep layers of the microvessels because of the wide focal distance range. Therefore, by using both a video system and a still camera, the changes in blood flow can be evaluated not only in the superficial layer but also in the deep layer of the capillary network.

By using a color image analyzer, the areas of the capillary network both in the superficial and deep layers were evaluated. However, there are some difficulties to overcome with this procedure: the photos were affected by various conditions such as lighting, imprinting and positioning of the bulbar conjunctiva. To get over these difficulties, 16 photos were taken in each case. The most appropriate photos were in terms of similar conditions then chosen for a comparative analysis. The changes of the capillary network areas evaluated in this manner were quite minimal in case of controls (Table III), reflecting the reliability of this method. By this new method, then the different mode of action between Keishi-bukuryo-gan and Oren-gedoku-to became apparent.

Concerning the reasons for the different actions between these two drugs, two possibilities can be entertained: the first one is that Oren-gedoku-to has different effects on the internal and external carotid arteries, and the other is that there may be auto-regulation systems of microvessels that are different from each other in regard to superficial and deep layers of bulbar conjunctiva.

The indicative signs of Oren-gedoku-to have traditionally been described as a red face, psychological irritability and hyperemia of bulbar conjunctiva,⁸⁾ indicating that this formula has the effect to decrease the blood flow of the facial skin, which is under the influence of the external carotid artery. Therefore, this clinical description would support the former possibility.

Recently the effectiveness of Oren-gedoku-to on symptoms associated with the cerebro-vascular type of dementia has been reported. Araki¹⁾ reported that psychiatric symptoms in 16 of 32

cases with this disorder improved following the administration of Oren-gedoku-to extracts for 12 weeks. Kogure²⁾ revealed that this prescription also increased the blood flow in ischemic brain tissue (especially in the hippocampal area) induced by the artificially occluded internal carotid artery.

In this study, it is newly suggested that both Oren-gedoku-to and Keishi-bukuryo-gan increased the microcirculation of the optic artery area which is derived from the internal carotid artery, although their modes of action were different from each other.

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和文抄録

黄連解毒湯のヒト眼球結膜の微小循環に対する効果を解明する目的で、10人の健常人（男性、20-42歳）にビデオ顕微鏡システムの観察録画下に、本方剤の急性負荷試験を行なった。TVモニター上の再生録画により、微小循環の血流速度および血管径を測定し、血流量を算出した。我々は、先に桂枝茯苓丸の微小循環に対する効果を報告したが、今回本方剤との作用機序の比較検討の為、桂枝茯苓丸の負荷試験も行なった。その結果、黄連解毒湯は表層血管の血流を低下させ、深層血管の血流を増加させ、これに対して、桂枝茯苓丸は表層と深層両部で血流を増加させた。この相違の発現機序は不明であるが、1つの可能性として、眼球表層の血流が内頸・外頸両動脈の供給を受けているのに対して、深層の血流は主として内頸動脈由来の眼動脈より供給されているという、解剖学的差異によるものと考案した。

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