Hemorheological studies of "oketsu" syndrome

－Erythrocyte aggregation in "oketsu" syndrome－

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Abstract

In order to elucidate the hemorheological characteristics of the "oketsu" syndrome, 24 patients with multiple lacunar infarction were evaluated. According to the diagnostic criteria presented by Terasawa *et al.*, the 24 patients were divided into three groups, a non- "oketsu" group, a mildly affected group and a severely affected group. Blood viscosity, erythrocyte aggregability and plasma fibrinogen level were measured in each group. Only in the severely affected group was there a significant increase in the corrected values of whole blood viscosity (CWBV) at a low shear rate (19.2sec^{-1}), erythrocyte aggregability and fibrinogen level as compared to the non "oketsu" group. In the mildly and severely affected group, there was a significant increase in the CWBV at a high shear rate (384.0 sec^{-1}). No significant difference was found in plasma viscosity among the three groups. In patients with a severe "oketsu" state, the severity of the state correlated with erythrocyte aggregability but not with the fibrinogen level. These results suggested that erythrocyte deformability is decreased in the mild and severe "oketsu" state, and that erythrocyte aggregability is increased in the severe "oketsu" state, moreover, the acceleration of erythrocyte aggregability in the severe "oketsu" state was due not only to elevation of the fibrinogen level but also due to pathophysiological disturbance on the erythrocyte membrane surface, such as reduced negative charge or increased membrane adhesiveness.

Key words "oketsu" syndrome, blood viscosity, erythrocyte aggregability, hemorheology.

Abbreviations CWBV, the corrected values of whole blood viscosity; DEA, maximum diameter of the column of intravascular erythrocyte aggregation; IEA, intravascular erythrocyte aggregation; Keishi-bukuryo-gan (Gui-Zhi-Fu-Ling-Wan), 桂枝茯苓丸; oketsu (Yu-Xue), 瘀血; S10, erythrocyte aggregation index.

Introduction

Although mortality due to cerebrovascular disease is decreasing, its morbidity is increasing as the population ages.[1] Japanese - Oriental (Kampo) medicine is attracting much interest in the treatment of cerebrovascular disease. At present, Kampo medicines are increasingly in use, however, most are administered according to a western medical diagnosis.[2] Ideally, Kampo medicines should be administered according to a Kampo medical diagnosis, using the "treatment according to Sho" advocated in the Classics. The term "Sho" means a conformation of signs and symptoms interpreted by Kampo pathology.

The subject of the present study, "oketsu", is a Kampo medical pathology defined by Shibazaki as "the state in which the blood is, for some reason, static and does not flow smoothly."[3] We

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used the "oketsu" diagnostic criteria developed by the Science and Technology Agency research group in response to a need for standardization in "oketsu" pathology research.[4] These criteria have already been used in many studies concerning microcirculatory dysfunction and hematological disturbances that occur in "oketsu."[5,6] Tosa *et al.* determined that an anti- "oketsu" prescription (Keishi-bukuryo-gan) improves the neurological symptoms of patients with cerebrovascular disease. They concluded that the improvement was due to the improvement in microcirculation promoted by the prescription (decreased blood viscosity and improved microcirculation in the bulbar conjunctiva).[7] Their study also demonstrated the existence of both a group of patients who did and a group who did not respond to the anti-"oketsu" prescription. They showed, in other words, that the severity of "oketsu" pathology varies among patients with cerebrovascular disease and that the severity of microcirculatory dysfunction correlates with the severity of "oketsu" pathology.

In the present study, patients with multiple old lacunar infarction were studied to determine how "oketsu" pathology is related to blood viscosity, bulbar conjunctival microcirculation, and erythrocyte aggregation (a factor which contributes to blood viscosity). The present study was undertaken in an attempt to elucidate the hemorheological characteristics of the blood in the "oketsu" syndrome.

Subjects and Methods

Subjects : Twenty-four patients (20 males, 4 females) with multiple old lacunar infarction who visited the Department of Japanese Oriental

Table I Clinical characteristics of patients with multiple lacunar infarcts.

Patient no.	Age years	Sex M/F	Signs and/or symptoms	Complication
1	75	M	right hemiplegia	DM
2	71	M	Parkinsonism	(-)
3	63	F	(-)	DM
4	71	M	dysarthria	IHD
5	69	M	right hemiplegia	DM HT
6	76	M	left leg numbness	(-)
7	74	M	left leg numbness	IHD
8	42	M	dysarthria, right hemiplegia	HT
9	65	M	dysarthria	(-)
10	71	M	(-)	DM HT
11	58	M	left paresthesia, left hemiplegia	(-)
12	67	M	(-)	(-)
13	63	F	(-)	HL
14	53	M	right hemiplegia	(-)
15	52	M	(-)	(-)
16	66	M	(-)	(-)
17	64	M	(-)	(-)
18	64	F	(-)	HL HT
19	66	F	(-)	DM HL HT
20	75	M	left hemiplegia	DM
21	44	M	left hemiplegia	HT
22	66	M	left hemiplegia	HL HT
23	67	M	Parkinsonism	(-)
24	61	M	left arm pain	(-)

HL ; hyperlipidemia, HT ; hypertension, IHD ; ischemic heart disease, DM ; diabetes mellitus

(Kampo) Medicine, Toyama Medical and Pharmaceutical University, were examined in this study. The characteristics (age, sex, signs and/or symptoms, and complications) of each patient are listed in Table I.

According to the diagnostic criteria presented by Terasawa *et al.*,[4] the 24 patients were divided into three groups, a non- "oketsu" group (n=5, "oketsu" score was 20 points and less), a mildly affected group (n=8, "oketsu" score was 21 points and above) and a severely affected group (n =11, "oketsu" score was 40 points and above).

Blood samples: To determine whole blood viscosity, plasma viscosity and erythrocyte aggregability, 14 ml of blood was withdrawn from the cubital vein into a siliconized glass tube containing ethylene-diamine-tetra acetic acid-2Na (1.5 mg/ml). Each sample was divided into three parts, one for measuring whole blood viscosity and the hematocrit, a second for measuring plasma viscosity, and the other for examining erythrocyte aggregability. Every blood sample was taken in the morning after overnight fasting and examined within at least three hours after sampling.

Measurement of viscosity: Viscosities of whole blood and plasma were measured by the cone-plate rotational viscometer (Bio-rheolizer, Tokyo Keiki Co., Ltd., TOKYO, JAPAN). The measurements were caried out at a temperature of 37°C and a cone angle of 1° 38′.

Measurement of the whole blood viscosity was carried out at three different shear rates (19.2 sec^{-1}, 76.8 sec^{-1} and 384.0 sec^{-1}). The values of whole blood viscosity (apparent viscosity) were corrected to a standard hematocrit value of 45 % using one of the following equations.[6]

#1) 19.2 sec^{-1}, $\log_{10}\eta_{45}=\log_{10}10\eta+0.0160\times(45-Ht)$

#2) 76.8 sec^{-1}, $\log_{10}\eta_{45}=\log_{10}10\eta+0.0111\times(45-Ht)$

#3) 384.0 sec^{-1}, $\log_{10}\eta_{45}=\log_{10}10\eta+0.0113\times(45-Ht)$

η, apparent viscosity (cP); Ht, hematocrit(%)

Plasma viscosity was measured at only one point of shear rate (384.0 sec^{-1}).

Measurement of erythrocyte aggregability: A SEFAM™ Erythro-aggregometer (SEFAM Co., Ltd., VANDOEUVRE LES NANCY CEDEX, FRANCE), a rotating viscosimeter with laser light backscattering was used to measure red cell aggregates (Fig.1). The whole blood sample was transfused into the space between the inner and the outer cylinder. The blood sample was sheared for 10 seconds at high shear rate (600 sec^{-1}). The erythrocytes deaggregated and became oriented to the flow. The backscattered light level was defined as the rDO at this time. After the shear stress was discontinued abruptly, the erythrocytes became disoriented and a rapid increase in the backscattered light was recorded. The maximum light level was noted as the rDD (=1). The progressive decrease in the intensity of the backscattered light is said to be related to the formation of "rouleaux" and "rouleaux-networks." The light intensity curves were displayed on the monitor.

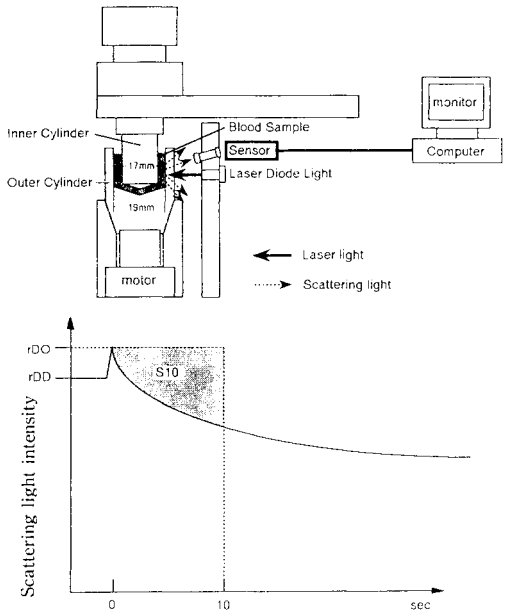


Fig. 1 SEFAM™ Erythro-aggregometer

The erythrocyte aggregation index (S10) was calculated from the relative area above the light intensity curve during the first 10 seconds after shear stress.[8] The measurements were made with the hematocrit adjusted to 40 % with autologous plasma and at a stable temperature of 37°C.

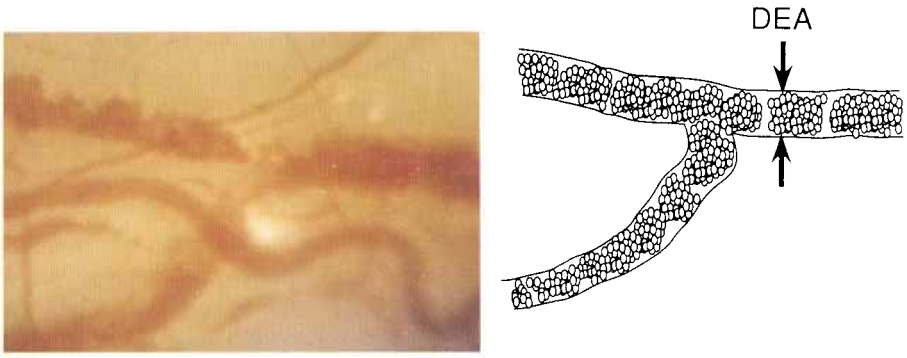


Fig. 2 DEA
The maximum diameter of the column of intravascular erythrocyte aggregation (DEA) was defined as the maximum diameter of the largest venule in which intravascular aggregation (IEA) was observed using a video-microscope system.

Observation of the bulbar conjunctiva microcirculation: Intravascular erythrocyte aggregation (IEA) in the plural venule of the bulbar conjunctiva was observed using a video-microscope.[9, 18]

The maximum diameter of the erythrocyte aggregation column (DEA) was defined as the maximum diameter of the largest venule in which IEA was observed. A typical case of the DEA is presented in Fig. 2.

Measurement of the hematocrit: The hematocrit was measured by microcentrifugation at 12500 G for 10 minutes.

Measurement of plasma protein concentration: The remainder of the blood sample was examined for total protein, albumin, globulin and plasma fibrinogen concentration. Plasma fibrinogen was measured by the thrombin time method.

Statistical analysis: The grouped data are presented as the mean±standard deviation. Statistical comparisons were made using the Mann-Whitney test. Correlations between the DEA and S10 were calculated using Pearson's correlation analysis. For every statistical test, the level of significance was $p<0.05$.

Results

Comparison of the corrected values of whole blood viscosity among the three groups

The corrected values of whole blood viscosity (CWBV) in the three groups are listed in Table II. The results indicate that the CWBV at low shear rate (19.2 sec^{-1}) in the severely affected group was significantly elevated in comparison to the other groups, however, the CWBV at a low shear rate did not differ significantly between the non "oketsu" and mildly affected group, and that the

Table II Comparison of corrected whole blood viscosity among the three groups.

	non-"oketsu" group (n=5)	mildly affected group (n=8)	severely affected group (n=11)	
Corrected whole blood viscosity				
shear rate				
19.2 sec^{-1}	7.19±0.95	7.20±0.57	8.23±0.82[a,b]	(cP)
76.8 sec^{-1}	4.88±0.35	5.17±0.15[a]	5.45±0.38[a,b]	(cP)
384.0 sec^{-1}	3.71±0.11	3.98±0.21[a]	4.15±0.20[a]	(cP)

The values are expressed as the mean±standard deviation.
[a] $p<0.05$: differs significantly from the non-"oketsu" group.
[b] $p<0.05$: differs significantly from the mildly affected group.

Table III Comparison of S10 or plasma viscosity among the three groups.

	non-"oketsu" group (n=5)	mildly affected group (n=8)	severely affected group (n=11)	
S10	22.00±2.51	24.84±2.85	28.69±3.00[a,b]	
Plasma viscosity (shear rate=384.0 sec^{-1})	1.32±0.08	1.37±0.07	1.50±0.17	(cP)

The values are expressed as the mean±standard deviation.
[a] $p<0.01$: significantly differs from the non-"oketsu" group.
[b] $p<0.01$: significantly differs from the mildly affected group.

CWBV at high shear rate (384.0 sec^{-1}) in the mildly and severely affected groups was significantly elevated in comparison to the non- "oketsu" group.

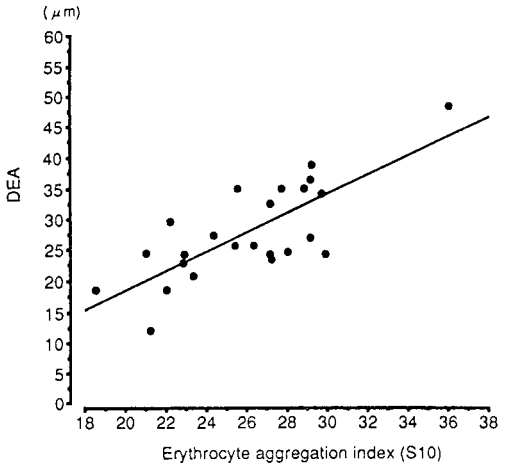
Comparison of the erythrocyte aggregation index (S10) and the plasma viscosity among the three groups

From the results which show the CWBV at a low shear rate in the severely affected group to be significantly higher than that in the other groups, it was deduced that erythrocyte aggregability and plasma viscosity were the important determinants of the severe "oketsu" state. Accordingly the erythrocyte aggregation index (S10) and the plasma viscosities were compared among the three groups. (Table III)

The S10 of the severely affected group was elevated significantly compared to that of any other group. The plasma viscosity, however, did not differ significantly among the three groups.

Correlation between the DEA and the S10

In order to determine whether the DEA can be used to quantitate erythrocyte aggregability, the relationship between DEA and S10 was evaluated (Fig.3). The DEA was correlated strongly with the S10.



Simple linear regression
y=1.552×-11.708
r=0.764
p=0.0001

Spearman's corr. coef.
rs=0.668
p=0.0014

Fig. 3 Correlation between DEA and S10.

Comparison of the DEA among the three groups

The DEA measured in each group are listed in Table IV. The DEA in the severely affected group was significantly higher than that in the

Table IV Comparison of DEA among the three groups.

	non-"oketsu" group (n=5)	mildly affected group (n=8)	severely affected group (n=11)	
DEA	21± 5	27± 4	34± 7[a,b]	(μm)

The values are expressed as the mean±standard deviation.
[a] $p<0.005$: significantly differs from the non-"oketsu" group.
[b] $p<0.05$: significantly differs from the mildly affected group.

Table V Comparison of plasma protein concentration among the three groups.

	non-"oketsu" group (n=5)	mildly affected group (n=8)	severely affected group (n=11)	
Fibrinogen	225.0±57.2	245.6±27.4	339.9±52.5[a,b]	(mg/dl)
Total protein	7.16±0.54	7.46±0.99	7.15±0.68	(g/dl)
Albumin	4.45±0.21	4.76±0.54	4.50±0.38	(g/dl)
Globulin	2.70±0.41	2.70±0.51	2.65±0.64	(g/dl)

The values are expressed as the mean±standard deviation.
[a] $p<0.05$: significantly differs from the non- "oketsu" group.
[b] $p<0.01$: significantly differs from the mildly affected group.

Table VI Correlation between "oketsu" score and plasma proteins or S10 in the severely affected group. (n=11)

	rs	p.value
Erythrocyte aggregability		
S10	0.830	**0.0087**
Plasma protein concentrations		
Fibrinogen	0.350	0.2662
Total protein	0.300	0.3391
Albumin	-0.170	0.5823
Globulin	0.320	0.3143

Spearman's Corr. Coef.

other groups. However, there was no significant difference between the non-"oketsu" and mildly affected group, in the DEA.

Comparison of the plasma protein concentration among the three groups

The fibrinogen concentration was significantly higher in the severely affected group. However, the albumin and globulin concentration did not differ significantly among the three groups. (Table V)

Correlation between the "oketsu" score and both the S10 and plasma protein concentration in patients with the severe "oketsu" state

The "oketsu" score correlated significantly with the S10 but not with albumin, globulin or fibrinogen concentration (Table VI).

Discussion

Previous studies have demonstrated the hematological and hemorheological abnormalities which accompany the "oketsu" state.[5,6] However, the patients studied suffered from many other concurrent diseases and no study has reported on subjects with single disease entity. Hemorheological parameters vary among diseases.[10] Consequently, in order to study the characteristics of the "oketsu" syndrome, patients with single disease entity should be selected as subjects. In this study patients with multiple lacunar infarction, a hemorheological disturbance[11-13] and the "oketsu" state[14] were examined. The present study shows that even in patients with only multiple lacunar infarction, there is variation in the severity of the "oketsu" state. The relationships between "oketsu" pathology and the hemorheological characteristics of blood were studied.

Hemorheological studies of the "oketsu" syndrome have elucidated that the corrected value of whole blood viscosity in the "oketsu" syndrome patient is significantly higher than that of non-"oketsu" subjects.[6] The present study shows that only in the severe "oketsu" state, there is a significant increase in the CWBV at low shear rate and that in mild and severe "oketsu" state, there is a significant increase in the CWBV at a high shear rate.

The most important determinant of whole blood viscosity are the hematocrit, plasma viscosity, erythrocyte aggregability and erythrocyte deformability.[15]

The CWBV adjusts for the effect of the hematocrit and in the present study the shear rate and temperature are held constant. Consequently, the variation in the CWBV must be due to differences in plasma viscosity, erythrocyte ag-

gregability and erythrocyte deformability. In particular, at a low shear rate, erythrocyte aggregability, and at a high shear rate, erythrocyte deformability, affect strongly the CWBV.[16]

No significant difference was found in plasma viscosity between the non-"oketsu" group and the mildly affected group nor between the mildly and severely affected groups. Although the severely affected group had a significantly increased erythrocyte aggregation index (S10), there was no significant difference in the S10 between the non-"oketsu" and mildly affected groups.

These results suggest that the increase in CWBV seen in the mild "oketsu" sate result from a decrease in erythrocyte deformability and that the influence of erythrocyte aggregation increases with the increasing severity of the "oketsu" state.

Terasawa *et al.* showed that the rate of intravascular erythrocyte aggregation in the the bulbar conjunctival microcirculation of patients with the "oketsu" syndrome is increased.[9] However, many studies of intravascular erythrocyte aggregation in the microcirculation of bulbar conjunctiva have shown that because of the effects of branching turbulence and blood flow velocity, it is difficult to quanitate the increase in erythrocyte aggregation.[17,18]

In the present study, an attempt was made to quantitate the level of erythrocyte aggregation by measuring the maximum internal diameter of the microvessels in which erythrocyte aggregation occurred. This measurement was defined as the maximum diameter of the column of the intravascular erythrocyte aggregation (DEA). To determine whether or not the DEA reflects correctly the IEA, the correlation between the DEA and the S10 was determined. It was found that DEA correlates significantly with the S10, suggesting that the DEA may be used as an index of *in vivo* erythrocyte aggregability. This may be due to the fact that because the velocity of plasma increases with vessel size,[19] erythrocytes are deaggregated by the shear stress of the velocity.[10] Namely, it is inferred that when IEA is observed in large vessels, erythrocyte aggregability may be elevated.

In this study, the DEA in the severely affected group was significantly higher than that in the other groups, suggesting that acceleration of erythrocyte aggregation contributes the severe "oketsu" state *in vivo*.

Factors contributing to erythrocyte aggregability include: erythrocyte adhesion, intercellular repulsion created by negative charges on the erythrocyte membrane surface, and plasma proteins, the most important of which is fibrinogen, a bridging molecule.[20,21] In each patient, the S10 correlated strongly with the fibrinogen level but not with total protein, albumin or the globulin level. The fibrinogen level is elevated significantly only in the severely affected group. However, in patients with severe "oketsu" state, the fibrinogen level does not correlate with the severity of the "oketsu" state. This suggests that in the severe "oketsu" state, there is not only an elevation of the fibrinogen level but also a pathophysiological disturbance on the erythrocyte membrane surface, such as reduced negative on the erythrocyte surface charge or increased membrane adhesiveness.

和文抄録

瘀血症候群における血液レオロジー的特性を明らかにするために，multiple lacunar infarction の患者24例について検討した。寺澤らの瘀血診断基準に従って24例の患者を非瘀血群5例，軽度瘀血群8例，重度瘀血群11例の三群に分類し，各群で血液粘度，赤血球集合能，フィブリノーゲン値を測定した。非瘀血群に対して重度瘀血群においてのみ，ずり速度19.2 sec^{-1} の低ずり速度での補正全血粘度，赤血球集合能およびフィブリノーゲン値の有意な上昇を認めた。軽度および重度瘀血群の両者においてずり速度384.0 sec^{-1} の高ずり速度で補正全血の有意な上昇を認めた。血漿粘度は三群間で有意差はなかった。また，重度瘀血群内では瘀血状態が重症化してもフィブリノーゲン値の上昇は見られなかった。

これらの結果より，軽度瘀血状態では赤血球変形能が低下し，重度瘀血状態に至って赤血球変形能の低下に加え赤血球集合能が亢進することが示唆された。また，重度瘀血状態における赤血球集合能の亢進はフィブリノーゲン値の上昇のみならず赤血球膜表面の陰性荷電の低下や赤血球膜の粘着性の亢進といった赤血球膜の病態生理学的変化が関与している

ことが示唆された。

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