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Erythrocyte deformability in "oketsu" syndrome and its relations to erythrocyte viscoelasticity

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Abstract

The deformability and aggregability of erythrocytes are important factors for the elevation of blood viscosity. Previously, we investigated the hemorheological abnormalities of the "oketsu" syndrome, and found a correlation between the severe "oketsu" state and the acceleration of erythrocyte aggregability. In the present study, 25 patients with multiple lacunar infarction were evaluated to determine the relationship between the "oketsu" state and erythrocyte deformability. According to the diagnostic criteria of Terasawa *et al.* ("oketsu" score), the patients were divided into three groups, a non-"oketsu" group, a mildly affected group and a severely affected group. Erythrocyte aggregability, erythrocyte deformability, elastic modulus of the erythrocyte membrane, and adenosine triphosphate (ATP) content in erythrocytes were measured in each subject. Compared with the non-"oketsu" group, erythrocyte aggregability was significantly accelerated only in the severely affected group, and erythrocyte deformability was significantly depressed in both the mildly and severely affected groups. There was a significantly positive correlation between the "oketsu" score and the elastic modulus of the erythrocyte membrane. ATP content in erythrocytes, one of the important factors for preservation of the erythrocyte membrane structure, was significantly negatively correlated with the "oketsu" score. These results suggested that not only erythrocyte aggregability but also erythrocyte deformability are related to the "oketsu" state, and that the deterioration of erythrocyte deformability in the "oketsu" state is due to the decreased erythrocyte viscoelasticity caused by the lower intracellular ATP concentration.

Key words "oketsu" syndrome, hemorheology, erythrocyte aggregability, erythrocyte deformability, erythrocyte viscoelasticity, adenosine triphosphate.

Abbreviations ATP, adenosine triphosphate ; CWBV, corrected whole blood viscosity ; HDL, high density lipoprotein ; MCV, mean corpuscular volume ; MCHC, mean corpuscular hemoglobin concentration ; oketsu, (Yu-Xue), 瘀血 ; PBS, phosphate buffered saline.

Introduction

Hemorheology, the science of the flow behavior of blood, has become important in various clinical fields.[1)] Several authors have reported on the relationship between cerebrovascular diseases and hemorheological abnormalities.[2,3)] Hemorheology is dependent on blood viscosity, which is influenced by hematocrit, plasma viscosity, erythrocyte aggregability and erythrocyte deformability.[4)] Especially, the disorder of erythrocyte deformability was not only closely related to cerebrovascular attack,[5,6)] but also reflected the patient's prognosis,[7)] including dementia.[8)]

"Oketsu", blood stasis or stagnant syndrome, is

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Table I Diagnostic criteria for "oketsu" syndrome

Symptoms	Score Male	Score Female	Symptoms	Score Male	Score Female
Dark shade around the eyes	10	10	Tenderness of leff navel region	5	5
Pigmentation over the face	2	2			
Rough skin	2	5	Tenderness of right navel region	10	10
Purple discoloration of lips	2	2	Tenderness under the navel region	5	5
Purple discoloration of gums	10	5			
Purple discoloration of tongue	10	10	Tenderness of iliocoecal region	5	2
Telangiectasis, vascular spider	5	5			
Susceptibility to subcutaneous bleeding	2	10	Tenderness of hypochondrial region	5	5
Redness of palms, palmar erythema	2	5	Tenderness of sigmoid region	5	5
			Hemorrhoids	10	5
			Dysmenorrhea		10

Evaluation : 20 points and less : non-"oketsu" state.
21 points and above : mildly affected "oketsu" state.
40 points and above : severely affected "oketsu" state

one of the pathological physiological concepts existing only in Chinese medicine.[9] This pathological state refers to a state of insufficient blood-circulation and blood stasis.[10] For the evaluation of "oketsu" state, this day, the evaluation criteria presented by Terasawa *et al.*[9] ("oketsu" score) is used (Table I).

Recently, we reported that patients with "oketsu" syndrome suffered more multiple lacunar infarction than non- "oketsu" patients.[11] Previously, we also reported that "oketsu" syndrome is closely correlated with abnormalities of the microcirculation, based on observations of blood flow of the bulbar conjunctiva,[10] and also such hemorheological abnormalities as the elevation of blood viscosity and erythrocyte aggregability.[12, 13] However, the disorder of erythrocyte aggregability was found only in patients with a severe "oketsu" state, and was not so serious when the "oketsu" state was mild.[13] Thus, it was presumed that some other factors was related to the elevation of blood viscosity in the mildly affected "oketsu" state.

In addition to erythrocyte aggregability, erythrocyte deformability is also an important factor to affect blood viscosity. The present study was performed to clarify the relationship between erythrocyte deformability and the "oketsu" state. Further, we examined the relation between the "oketsu" state and such factors affecting erythrocyte deformation as cell surface area to volume ratio, intracellular viscosity and cell membrane viscoelaticity.

Subjects and Methods

Subjects : Twenty-five male patients (range 44-79 yrs, mean 64.4±10.7 yrs) with multiple lacunar infarction who visited the Department of Japanese Oriental (Kampo) Medicine, Toyama Medical and Pharmaceutical University Hospital, were examined. Age-matched normal control group (n=11, range 50-73 yrs, mean 61.8±7.8 yrs) consisted of healthy men. Based on the evaluation criteria presented by Terasawa *et al.* ("oketsu" score),[9] the patients were divided into three groups, a non-"oketsu" group (n=8, "oketsu" score 20 points or less), a mildly affected group (n=10, "oketsu" score 21 points or higher, but less than 40 points), and a severely affected group (n=7, "oketsu" score 40 points or higher). The subjects' characteristics are described in Table II. The "oketsu" score was determined by two specialists in Kampo medicine before measurement of the hemorheological parameters.

Blood samples : To determine erythrocyte aggregability, erythrocyte deformability, erythrocyte viscoelasticity and adenosine triphosphate (ATP) content in erythrocytes, 7 ml blood, anticoagulated in

Table II Comparison of clinical features among the three groups

	Non-"oketsu" group (n=8)	Mildly affected group (n=10)	Severely affected group (n=7)	
Age[a]	63.4±8.4	65.1±11.5	64.4±13.3	N.S.
Neurological symptoms[b] and signs	2 (25.0 %)	3 (30.0 %)	2 (28.6 %)	N.S.
Complications[b]				
Hypertension	2 (25.0 %)	3 (30.0 %)	2 (28.6 %)	N.S.
Diabetes	3 (37.5 %)	2 (20.0 %)	1 (14.3 %)	N.S.
Hyperlipidemia	1 (12.5 %)	2 (20.0 %)	1 (14.3 %)	N.S.
Ischemic heart disease	1 (12.5 %)	1 (10.0 %)	2 (28.6 %)	N.S.

[a]The values are expressed as the mean±standard deviation. Statistical analysis was done by Kruskal-Wallis test.
[b]The data are expressed as the number of patients (%). Statistical analysis was done by chi-square for independence.
N.S. : not significant

EDTA-2Na (1.5 mg/ml), and 5 ml heparinized blood, were withdrawn from the cubital vein. Each blood sample was taken in the morning after overnight fasting and examined within four hours after sampling. The heparinized blood was used for ATP measurement. The blood anticoagulated in EDTA-2Na was used for measuring the other parameters.

Measurement of erythrocyte aggregability : Erythrocyte aggregability was measured by SEFAM™ Erythro-aggregometer (SEFAM Co., Ltd., VANDOEUVRE LES NANCY CEDEX, FRANCE), a rotating viscometer with laser backscattering, according to the method in our previous report.[13]

Measurement of erythrocyte deformability : The apparatus for measurement of erythorcyte deformability was prepared, with some modification, according to Reid's filtration method (Fig. 1).[14] 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). The washed erythrocytes, aspirated from the middle of the packed erythrocyte column, were resuspended in isotonic PBS to a final concentration of 15 %. Erythrocyte deformability was determined by measuring their ability to pass through a 5 μm pore filter (Nucleopore, Costar Co., Ltd., USA) under constant -10 cmH_2O pressure.

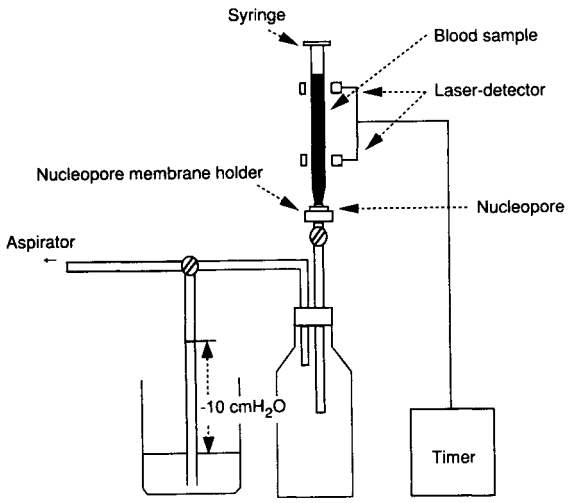

Fig. 1 The apparatus for measuring erythrocyte deformability (Erythrocyte filtration method). Packed erythrocytes are washed three times with isotonic phosphate buffered saline (PBS) (pH=7.4, 295 mOsm/kg). Washed erythrocytes are resuspended in isotonic PBC to a final 15 % concentration. Erythrocyte deformability is determined by maesuring their ability to pass through a 5 μm pore filter under constant negative pressure (-10 cmH_2O). In each filtration experiment, the time required for 400 μl of the 15 % red cell suspension to pass through the filter is determined.

In each filtration experiment, the time required for 400 μl of the 15 % red cell suspension to pass through the filter was determined. The deformability index was calculated as the average of eight repeated tests. A fresh filter was used for each measurement, and all measurements were performed at 25°C. Deformability experiments were performed in accordance with guidelines set by the International Committee for Standardization in Hematology, Expert Panel on Blood Rheology.[15]

Hematocrit (microhematocrit, 12,500×g for 10 min., in duplicate), total plasma protein, albumin, total plasma cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol and fasting plasma glucose were measured. The cell count of erythrocytes and hemoglobin concentration were also measured (Auto cell counter Celltac MEK-4500 ; Nihon kohden, Co., Ltd., Tokyo, Japan) to determine mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

Measurement of erythrocyte viscoelasticity : On the basis of Hochmuth's method,[16] erythrocyte viscoelasticity (i.e. elastic modulus of erythrocyte membrane) was determined by means of a parallel plate flow channel chamber (Fig. 2).

After plasma and buffy coat were removed from 7 ml blood with EDTA-2Na, the remaining packed erythrocytes were washed three times with isotonic PBS. The washed erythrocytes were resuspended in isotonic PBS to a final 0.1 % concentration. The erythrocyte suspension was injected into the parallel plate flow channel chamber at a constant shear rate via a DC infusion pump. Elongation of single erythrocyte attached to the flow channel's glass surface was observed with an inverted microscope and recorded with a CCD camera (MKC-385, Olympus Co., Ltd., Tokyo, Japan) (Fig. 3). For each blood sample, the elastic modulus of the erythrocyte membrane was calculated from the elongation of 10 point-attached cells according to Hochmuth's formula.[16]

Measurement of ATP content in erythrocytes : After plasma and buffy coat were removed from 5 ml heparinized blood, the remaining packed erythrocytes were washed three times with isotonic PBS at 4°C. ATP in the packed erythrocytes was extracted by the method of Yokoyama *et al.*[17] By using an ATP assay kit (LL-100-1, Toyo Ink Co., Ltd., Tokyo, Japan), luminescence was caused by reacting the extracted ATP on luciferase (firefly's luminescent enzyme). The degree of luminescence was measured with a luminescence reader (BLR - 301, Aloka Co., Ltd., Tokyo, Japan).

Statistical analysis : The data were presented as mean±standard deviation. Analyses of independence

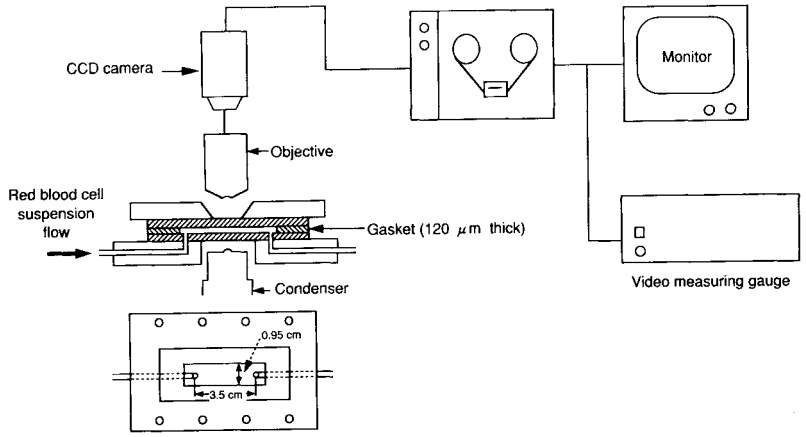

Fig. 2 System diagram of the parallel plate flow channel chamber and the video-microscope system. Erythrocyte suspension in 0.1 % hematocrit with isotonic PBC is injected into the parallel plate flow channel chamber 120 μm thick at a constant shear rate (100 ml/hr) by DC infusion pump. Elongation of single erythrocyte attached to the flow channel's glass surface is observed by inverted microscope and recorded using a CCD camera.

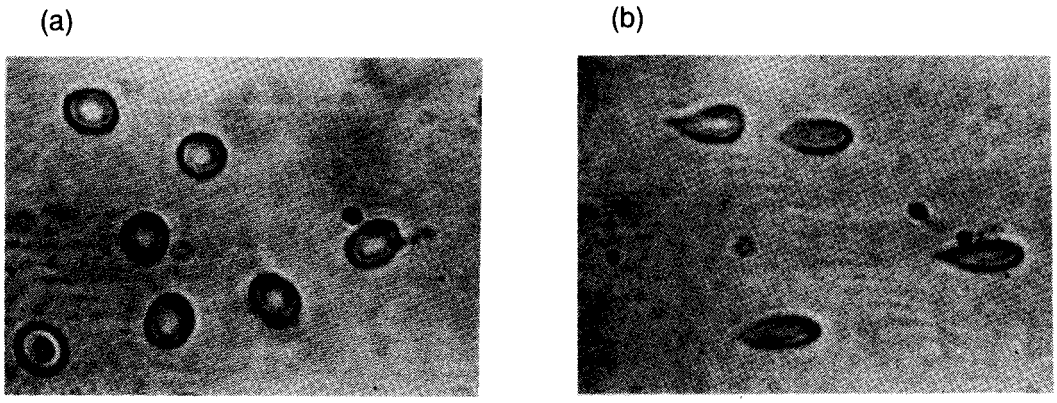

Fig. 3 Photomicrographs of erythrocytes without shear stress (a) and elongation of erythrocytes under shear stress (b).

were performed by chi-square test, and the strengths of relations were assessed by Spearman's rank correlation coefficients. Statistical comparisons were made using the Kruskal-Wallis test and Mann-Whitney test. For every statistical test, the level of significance was $p<0.05$.

Results

Comparison of laboratory data among the groups

Table III shows the laboratory data of the control and patients groups. There was no statistical significance in any value (hematocrit, total plasma protein, albumin, total plasma cholesterol, HDL-cholesterol, triglyceride and fasting plasma glucose) among the groups. Therefore, there was no significant difference in the clinical features among the groups to be classified according to severity of the "oketsu" state.

Comparison of MCV and MCHC among the groups

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) did not differ significantly among the groups (Table IV).

Comparison of erythrocyte aggregability and erythrocyte deformability among the groups (Fig. 4)

Erythrocyte aggregability in the severely affected group (28.7±3.7) was significantly higher than that in

Table III Comparison of laboratory data among the three groups

		Control group (n=11)	Non-"oketsu" group (n=8)	Mildly affected group (n=10)	Severely affected group (n=7)	
Hematocrit	(%)	45.0±2.9	43.4±4.0	45.0±3.9	44.2±3.9	N.S.
Total-protein	(g/dl)	7.1±0.5	7.4±0.2	7.5±0.6	7.2±0.6	N.S.
Alubumin	(g/dl)	4.3±0.3	4.5±0.2	4.3±0.2	4.2±0.4	N.S.
Total-cholesterol	(mg/dl)	189.8±39.3	217.3±33.8	194.8±38.1	194.4±47.9	N.S.
Triglyceride	(mg/dl)	129.1±62.6	174.6±52.9	125.9±74.6	153.9±79.1	N.S.
HDL-cholesterol	(mg/dl)	40.6±9.3	41.6±12.7	55.6±18.2	46.3±10.8	N.S.
Fasting plasma glucose	(mg/dl)	104.0±14.4	94.1±9.8	105.5±22.7	99.4±16.7	N.S.

The values are expressed as the mean±standard deviation. Statistical analysis was done by Kruskal-Wallis test.
N.S.: not significant

Table IV Comparison of MCV and MCHC among the three groups

	Control group (n=11)	Non-"oketsu" group (n=8)	Mildly affected group (n=10)	Severely affected group (n=7)	
Mean corpuscular volume (MCV) (μm^3)	94.1±4.4	95.9±4.4	98.3±8.0	101.4±6.1	N.S.
Mean corpuscular hemoglobin concentration (MCHC) (%)	33.1±0.8	33.0±1.1	32.8±1.4	32.5±0.6	N.S.

The values are expressed as mean±standard deviation. Statistical analysis was done by Kruskal-Wallis test.
N.S. : not significant

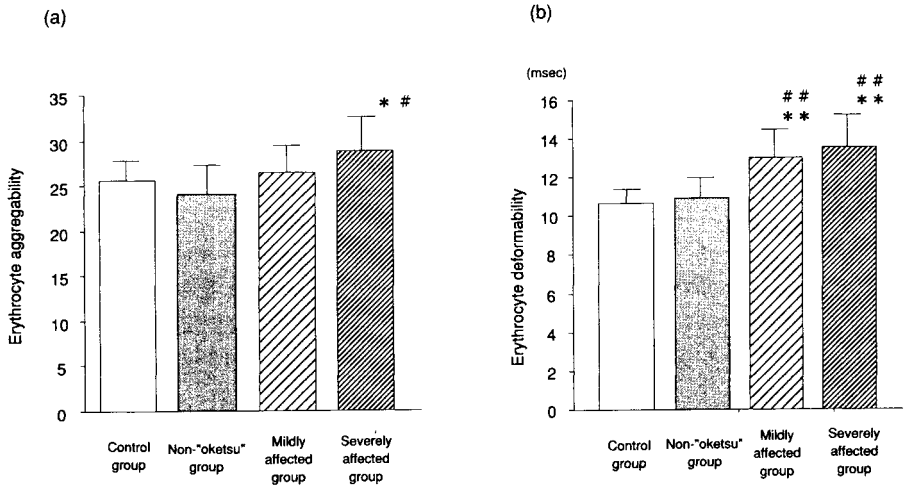

Fig. 4 Comparison of erythrocyte aggregability (a) and erythrocyte deformability (b) among the groups. The values are expressed as mean±S.D. Statistical analysis was done by Mann-Whitney test. $*p<0.05$: significant difference from non-"oketsu" group. $**p<0.01$: significant difference from non-"oketsu" group. $\#p<0.05$: significant difference from the control group. $\#\#p<0.01$: significant difference from the control group.

the non-"oketsu" group (24.0±3.2) ($p<0.05$), but there was no significant difference between the non-"oketsu" group and the mildly affected group (26.4±3.1).

Erythrocyte deformability in the severely affected group (13.6±1.6 msec) was also significantly higher than that in the non-"oketsu" group (11.0±1.0 msec) ($p<0.01$). Furthermore, there was also significant difference between the non-"oketsu" group and the mildly affected group (13.0±1.6 msec) ($p<0.01$).

Both the aggregability (25.2±2.5) and the deformability (10.9±1.5 msec) in the control group were close to those in the non-"oketsu" group.

Correlation between erythrocyte deformability, erythrocyte viscoelasticity, ATP content in erythrocytes and "oketsu" score of the subjects

Erythrocyte deformability was significantly positively correlated with the "oketsu" score (Fig. 5). Erythrocyte viscoelasticity also showed a significant positive correlation with the "oketsu" score. On the other hand, ATP content in erythrocytes had a significant negative correlation with the "oketsu" score (Table V).

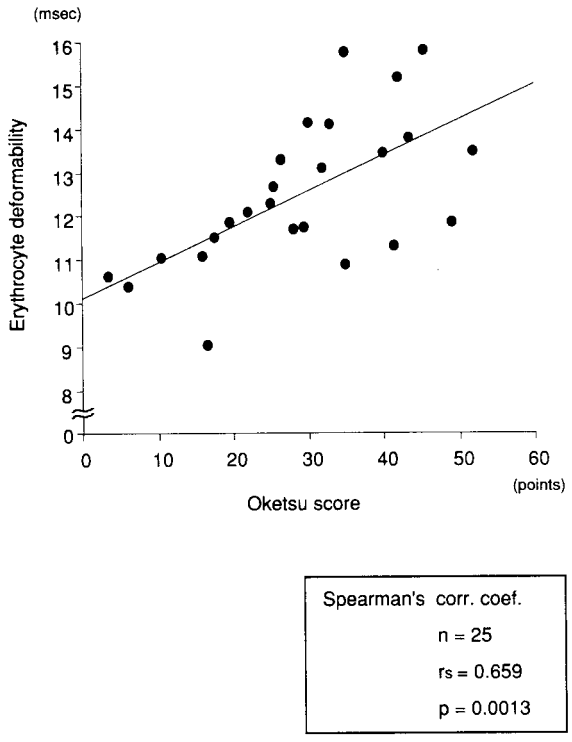

Fig. 5 Correlation between "oketsu" score and erythrocyte deformability.

Table V Correlation between "oketsu" score and erythrocyte viscoelasticity or ATP content in erythrocytes

	r_s	p-value
Elastic modulus of erythrocyte membrane	0.506	0.0131
ATP content in erythrocytes	-0.467	0.0223

(Spearman's Corr. Coef.)

Discussion

It is considered that the factors influencing whole blood viscosity are hematocrit, erythrocyte aggregability, erythrocyte deformability and plasma viscosity.[4] Our previous studies have demonstrated that plasma viscosity was not correlated with the "oketsu" state, but that an acceleration of erythrocyte aggregation was correlated to its severity.[13] As for the corrected whole blood viscosity (CWBV), which was adjusted to a value at 40 % hematocrit, a low shear (19.2 sec^{-1}) significantly increased both in mild and severe "oketsu" states, but a high shear rate (384.0 sec^{-1}) significantly increased in only the severe "oketsu" state.[13] It was reported that CWBV at a low shear rate was affected by erythrocyte deformability, and at a high shear rate by erythrocyte aggregability.[18] Therefore, it is expected that decreased erythrocyte deformability is related to both the mild and severe "oketsu" states.

In the present study, we examined the relationship between "oketsu" state and erythrocyte deformability, and that between "oketsu" state and the factors contributing to erythrocyte deformability. In the point of hemorheology, the non-"oketsu" patient group was similar to the normal control group. The erythrocyte deformability significantly decreased in both the mild and severe "oketsu" states, and it was suggested that the elevation of blood viscosity in the mild "oketsu" state is due to the worsening of erythrocyte deformability. On the other hand, a significant acceleration of erythrocyte aggregability was observed in the patients of the severe "oketsu" state. Therefore, it is suggested that not only erythrocyte aggregability but also deformability are related to the severity of the "oketsu" state.

It is generally considered that erythrocyte deformability is primarily affected by the following three factors : the ratio of cell surface area to volume, the intracellular viscosity and the cell membrane viscoelasticity.[18] The ratio of cell surface area to volume is dependent on the biconcave disk shape of the erythrocyte. Because all erythrocytes of the subjects in this study were normal shape and there was no difference in MCV among the patients' groups, it was considered that the ratio of cell surface area to volume was not correlated with the "oketsu" state. The intracellular viscosity of erythrocytes is dependent on the intracellular hemoglobin concentration. Therefore, the intracellular viscosity is assessed by MCHC.[18] The cell membrane viscoelasticity is evaluated by the modulus of the erythrocyte membrane. In the present study, MCHC was not correlated with the severity of the "oketsu" state. On the other hand, the modulus of

the erythrocyte membrane had a significant positive correlation, suggesting that the erythrocyte membrane viscoelasticity is lowered in patients of "oketsu" syndrome. Moreover, it is likely that the worsening of erythrocyte deformability in "oketsu" state is due to the worsening of the erythrocyte membrane viscoelasticity.

The erythrocyte membrane is composed of a lipid bilayer, which consists of free cholesterol, phospholipid and a cytoskeletal protein network (i.e., spectrin, actin, *etc.*).[18] Erythrocyte membrane viscoelasticity depends on these structures. ATP plays many important roles in the erythrocyte membrane, and especially in its viscoelasticity. Stability of the lipid bilayer is maintained by ATP-dependent aminophospholipid translocase.[19] Therefore, lipid mobility is influenced the intracellular ATP concentration.[20] Changes in the cytoskeletal protein structure results from a lowering of intracellular Ca^{++} concentration which can be due to a reduction of intracellular ATP concentration.[21] Moreover, because the strength of cytoskeletal protein bundling is also changed by the phosphorylation of cytoskeletal protein (i.e., spectrin) which is induced by ATP, the cytoskeletal architecture is influenced by the intracellular ATP concentration.[21, 22]

In the present study, ATP content in erythrocytes was significantly negatively correlated with the severity of the "oketsu" state. This suggests that the worsening of erythrocyte deformability in the "oketsu" state is due to a change in membrane composition caused by a decrease in intracellular ATP concentration.

In conclusion, our present study revealed that not only erythrocyte aggregability but also erythrocyte deformability is related to the "oketsu" state in patients with multiple lacunar infarction. Furthermore, the worsening of erythrocyte deformability in the "oketsu" state is suggested to be due to decreased erythrocyte viscoelasticity through decreased intracellular ATP concentration.

Previously, we observed that patients with "oketsu" syndrome had abnormalities of the microcirculation on the blood flow of their bulbar conjunctiva.[10] This study suggests that the abnormalities of the erythrocytes may be one of the causes of the microcirculation disorder in "oketsu" state. To prove this probability, it may be necessary to examine whether an improvement of erythrocyte deformability on patients has influence on recovering from "oketsu" syndrome. Moreover, an investigation must be made as to whether the vascular endotelial cells, one of the regulating factors to blood flow, plays an important part in microcirculation of "oketsu" state.

和文抄録

先に我々は瘀血病態における血液レオロジー異常について検討し，血液粘度上昇因子のうち赤血球集合能亢進が重度瘀血病態と関連していることを明らかにした。今回，正常対照群11名と多発性脳梗塞患者25例を対象に，血液粘度の影響因子である赤血球変形能と瘀血病態の関連性について検討し，さらに赤血球変形能を規定する因子との関連性についても検討した。多発性脳梗塞患者群25例は寺澤の瘀血診断基準に従って非瘀血群8例，軽度瘀血群10例，重度瘀血群7例の3群に分類し，赤血球集合能，赤血球変形能，赤血球膜弾性係数，赤血球ATP含量を測定した。その結果，正常対照群と非瘀血群は赤血球集合能，赤血球変形能に差はみられなかった。赤血球集合能亢進は重度瘀血病態にみられ，赤血球変形能低下は軽度及び重度瘀血病態に認められた。また，瘀血スコアと赤血球膜弾性係数の間に正の相関関数がみられた。さらに赤血球膜を構成する脂質組成および骨格蛋白間の結合に重要な役割を果たすATPについて検討したところ，瘀血スコアと赤血球ATP濃度との間に負の相関関数が認められた。以上の成績から，赤血球集合能のみならず赤血球変形能も瘀血病態と関連性を有すること，さらに瘀血病態における赤血球変形能の低下は赤血球ATP濃度の低下を介する赤血球膜粘弾性の低下が関連していることが示唆された。

References

1) Mokken, F. Ch., Kedaria, M., Henny, Ch. P., Hardeman, M. R. and Gelb, A. W. : The clinical importance of erythrocyte deformability, a hemorheological parameter. *Ann. Hematol.* **64**, 113-122, 1992.
2) Ernst, E., Matrai, A. and Marshall, M. : Blood rheology in patients with transient ischemic attacks. *Stroke* **19**, 634-636, 1988.
3) Coull, B. M., Beamer, N., Garmo, P., Sexton, G., Nordt, F., Knox, R. and Seaman, G. V. F. : Chronic blood hyperviscosity in subjects with acute stroke, transient ischemic attack, and risk factors for stroke. *Stroke* **22**, 162-168, 1991.
4) Shige, T. : Rheology of red cells. *J. Jpn. Physiol.* **44**, 187-198, 1982.

5) Resch, K. L., Ernst, E., Matrai, A., Buhl, M., Schlosser, P. and Paulsen, H. F. : Can rheologic variables be of prognostic relevance in arteriosclerotic diseases? *Angiology* **42**, 963-970, 1991.
6) Satoh, K., Tsuda, Y., Takahashi, T., Miyamoto, Y. and Matsuo, H. : Changes in hemorheological parameters in patients with acute cerebral infarction. *Jpn. J. Stroke* **15**, 30-39, 1993.
7) Takahashi, Y. : Red blood cell deformability in case of cerebrovascular diseases 2. clinical studies. *St. Marianna Med. J.* **11**, 60-73, 1983.
8) Honda, H., Kinoshita, M., Kamegai, M., Shimojo, S. and Miyahara, T. : A study of hemorheology in multiple infarcts and multi-infarct dementia. *Clin. Neurol.*, **26**, 625-630, 1980.
9) Terasawa, K., Shinoda, H., Imadaya, A., Tosa, H., Bandoh, M. and Satoh, N. : The presentation of diagnostic criteria for "oketsu" syndrome. *J. Jap. Soc. Oriental Medicine* (in Japanese) **34**, 1-17, 1983.
10) 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.
11) Hikiami, H., Kohta, K., Itoh, T., Shimada, Y. and Terasawa, K. : On the relationship between oketsu syndrome and distribution of multiple cerebral infarction on magnetic resonance imaging. *J. Jap. Soc. Oriental Medicine* (in Japanese with Eng. abst.) **46**, 547-554, 1996..
12) 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.
13) Kohta, K., Hiyama, Y., Terasawa, K., Hamazaki, T., Itoh, T. and Tosa, H. : Hemorheological studies of "Oketsu" syndrome Erythrocyte aggregation in "Oketsu" syndrome-. *J. Med. Pharm. Soc. WAKAN-YAKU* **9**, 221-228, 1992.
14) Reid, H. L., Barnes, A. J., Lock, P. J., Dormandy, J. A. and Dormandy, T. L. : A simple method for measuring erythrocyte deformability. *J. Clin. Pathol.* **29**, 855-859, 1976.
15) Bull, B. S., Chien, S., Dormandy, J. A., Kiesewetter, H., Lewis, S. M., Lowe, G. D. O., Meiselman, H. J., Shohet, S. B., Stoltz, J. F., Stuart, J. and Teitel, P. : Guidelines for measurement of blood viscosity and erythrocyte deformability. *Clin. Hemorheol.* **6**, 439-453, 1986.
16) Hochmuth, R. M., Mohandas, N. and Blackshear, Jr., P. L. : Measurement of the elastic modulus for red cell membrane using a fluid mechanical technigue. *Biophys. J.* **13**, 747-762, 1973.
17) Yokoyama, S. and Nose, Y. : Firefly luminescence assay method for ATP in erythrocyte. *J. Jap. Biochem. Soc.* (in Japanese) **39**, 46-49, 1967.
18) Shiga, T., Maeda, N. and kon, K. : Erythrocyte Rheology. *Cri. Rev. Oncol./Hematol.* **10**, 9-48, 1990.
19) Middlekoop, E., Van der Hoek, E. E., Bevera, E. M., Comfurius, P., Slotboom, A. J., Op den Kamp, J. A. F., Lubin, B. F., Zwaal, R. F. A. and Roelofsen, B. : Involvement of ATP-dependent aminophospholipid translocation in maintaining phospholipid asymmetry in diamide-treated human erythrocytes. *Biochim. Biophys. Acta* **981**, 151-160, 1989.
20) Mosior, M., Mikolajczak, A. and Gomulkiewicz, J. : The effect of ATP on the order and the mobility of lipids in the bovine erythrocyte membrane. *Biochim. Biophys. Acta* **1022**, 361-364, 1990.
21) Maeda, N. : Organization of the erythrocyte membrane skeleton and the rheology. *Medicina Philosophica* (in Japanese) 8 (suppl. 12), 965-971, 1989,
22) Backman, L. : Functional or futile phosphorus ?. *Nature* **334**, 653-654, 1988.