Improvement of Erythrocyte Deformability by Cholesterol-Lowering Therapy With Pravastatin in Hypercholesterolemic Patients

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Erythrocyte deformation is an important regulatory factor of the microcirculation. The present study was designed to examine whether erythrocyte deformability is altered in hypercholesterolemic patients and, if so, whether cholesterol-lowering therapy affects this parameter in these patients. The erythrocyte deformability of 37 hypercholesterolemic patients was evaluated before and after 1 year of therapy with pravastatin, an inhibitor of hepatic hydroxymethyl glutaryl coenzyme A reductase, under various shear stresses (4.7, 9.5, 23.6, 47.3, 118.1, and 236.2 dyne/cm^2) using laser diffractometry. At study entry, erythrocyte deformability under 4.7 and 9.5 dyne/cm^2 shear stress, which is actually observed in human vessels, was reduced compared with that in 20 age-matched normocholesterolemic subjects and was inversely correlated with serum cholesterol and low-density lipoprotein (LDL) cholesterol. Pravastatin therapy for 1 year, which reduced serum cholesterol from 288 ± 28 to 223 ± 20 mg/dL, significantly improved erythrocyte deformability by approximately 20%. There was a significant relation between the improvement of erythrocyte deformability and the reduction of serum cholesterol or LDL cholesterol. The results suggest that erythrocyte deformability is reduced in hypercholesterolemic patients, and that long-term cholesterol-lowering therapy can improve reduced erythrocyte deformability, which may contribute to the improvement of organ perfusion.

HYPERCHOLESTEROLEMIA is an important risk factor for coronary atherosclerosis.[1-4] Several mechanisms whereby hypercholesterolemia may cause ischemic heart disease have been proposed. A direct deposition of cholesterol promotes growth of the atherosclerotic plaque, thus leading to anatomical narrowing of the coronary artery.[5] In addition, hypercholesterolemia is associated with blunted endothelium-dependent vasodilation of the coronary microcirculation in animals and humans, which may impair myocardial perfusion in hypercholesterolemia.[6-10] On the other hand, an indirect mechanism whereby cholesterol influences coronary microcirculation is via its effects on hemorrheology. Hemorrheological factors such as blood viscosity, platelet activation state, and erythrocyte deformability may be affected by the level of blood cholesterol.[11,12]

Erythrocyte deformation is an important regulatory factor of the microcirculation in physiological and pathophysiological conditions.[13,14] There are several methods to measure erythrocyte deformability, but several common methods are dependent on factors that influence blood viscosity, such as the hematocrit, plasma viscosity, and cell aggregation. The filtration method, the most common, yields values influenced by cell-to-cell interactions in addition to factors that govern deformability.[15] This method reflects properties of the cell membranes and the geometry of the cell, but is little affected by internal viscosity.[15] The micropipette method also does not reflect internal viscosity.

Ektacytometry is now the most suitable method for evaluation of erythrocyte deformability, because this method is independent of factors other than erythrocyte deformability. However, detection of the diffraction pattern is technically complex. The oldest method for such detection is the measurement of diameters from photographs of the diffraction pattern. Bessis and Mohandas[16-17] and Groner et al[18] improved the original detection method by using a photosensor, and in 1986, Rasia et al[19] used a linear photosensor; however, both methods detected light intensities, not light intensity patterns. Recently, we[20] developed a sensitive and reproducible method to detect the light intensity pattern of diffraction images in ektacytometry by a linear image sensor and computer. Using this method, we examined whether erythrocyte deformability is altered in hypercholesterolemic patients and, if so, whether a 1-year cholesterol-lowering therapy with pravastatin, an inhibitor of hepatic hydroxymethyl glutaryl coenzyme A reductase, affects erythrocyte deformability in these patients. We also assessed the relationship between changes in erythrocyte deformability and the lipid profile during pravastatin therapy.

SUBJECTS AND METHODS

Patient Selection and Study Design

Between January 1992 and December 1995, we recruited 37 patients with primary hypercholesterolemia in our department. All patients underwent routine laboratory studies including assays of serum creatinine, blood urea nitrogen, blood cell counts, and fasting blood glucose, liver-function testing, urinalysis, chest roentgenogram, and electrocardiogram. We selected patients with primary hypercholesterolemia based on results of the laboratory tests. No patients had signs or symptoms of cardiac or renal failure, diabetes, or myocardial infarction. Before they were enrolled in this study, no patient had received any cholesterol-lowering drugs. Serum total and high-density lipoprotein (HDL) cholesterol and triglycerides were determined by the enzyme assay method. Low-density lipoprotein (LDL) level was calculated as total cholesterol − HDL cholesterol − (triglyceride/5).

After the baseline study was performed, 37 patients with hypercholesterolemia (total cholesterol, ≥250 mg/dL) were assigned to receive 10 mg/d pravastatin. If total cholesterol did not decrease to less than 240 mg/dL after 3 months, the dose of the drug was increased to 20 mg/d. Patients were allowed to take other antihypertensive drugs, which were not changed during the follow-up period. The results were compared

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with values in 20 age-matched healthy subjects with a serum cholesterol level of 220 mg/dL or less.

Deformability Measurement

Blood was drawn from patients treated with heparin, and processed immediately after collection. Then, 100 μL whole blood was suspended in 20 mL dextran solution (15% dextran dissolved in phosphate-buffered saline, pH 7.4).

Erythrocyte deformability was measured by rheocytometry, a laser diffractometric method that uses a flat glass flow cell and a 2-mW helium-neon laser (632.8 nm) as previously described.[21] In brief, erythrocytes are treated with various shear stresses (4.7, 9.5, 23.6, 47.3, 118.1, and 236.2 dyne/cm^2) whereby an erythrocyte suspension flows through the cell at various speeds controlled by a syringe pump (Truth A-II; Nakagawa-Seikoudo, Tokyo, Japan). The laser beam traverses the opening through the flow cell for the suspension flow, perpendicular to it, and the suspended particles, usually at least 2,000 erythrocytes, scatter the laser light to form a diffraction pattern 5 cm from elliptical as the erythrocytes are deformed in response to shear stress at increased flow speeds (Fig 1).

The diffraction pattern is then analyzed to evaluate cell deformation. The light-intensity pattern of the short and long axes of the diffraction pattern is assessed with a linear image sensor (S 2304-1024 Q; Hamamatsu Photonics, Hamamatsu, Japan) as previously described.[20] Results were expressed as the deformability index (DI), defined as DI = (L − W)/(L + W), in which L (length) and W (width) are the mean diameters measured along the long and short axes of the diffraction pattern from each set of eight measurements.[20]

The coefficients of variation for results obtained by our method were, respectively, 0.2%, 0.3%, 0.6%, 0.5%, 0.4%, and 0.5% with a shear stress of 4.7, 9.5, 23.6, 47.3, 118.1, and 236.2 dyne/cm^2, which is much smaller than the 1% limit recommended by the International Committee for Standardization in Haematology.[22]

Statistical Analysis

Mean ± SD values were determined for each variable studied. Parameters of hypercholesterolemic patients and normal controls were analyzed by the unpaired *t* test. Pretherapeutic and posttherapeutic values were compared using paired ANOVA and reexamined by the method of Greenhouse and Geisser.[23] Linear regression analysis was used to examine the relationship of the erythrocyte deformability index to various lipid parameters.

RESULTS

Table 1 lists clinical characteristics and deformability indices of erythrocytes under various shear stresses in normocholesterolemic control subjects and hypercholesterolemic patients. There were no significant differences in age, sex distribution, mean blood pressure, serum creatinine, and blood urea nitrogen between the two groups. Expectedly, total cholesterol and LDL cholesterol levels in hypercholesterolemic patients were markedly higher than in control subjects. Triglycerides in hypercholesterolemic patients tended to be higher than in control subjects, but the difference was not statistically significant. HDL cholesterol in hypercholesterolemic patients was not different from that in control subjects. The deformability index of erythrocytes under 4.7 and 9.5 dyne/cm^2 shear stress was reduced in hypercholesterolemic patients compared with control subjects. In contrast, the deformability indices under a shear stress of 23.6 dyne/cm^2 and greater were not different from values in control subjects.

The effect of pravastatin therapy for 1 year on the lipid profile and erythrocyte deformability index under various shear stresses is also shown in Table 1. Pravastatin therapy for 1 year clearly reduced total cholesterol and LDL cholesterol and slightly but significantly increased HDL cholesterol. In contrast, pravastatin therapy failed to reduce triglyceride levels even at 1 year. Pravastatin therapy for 1 year significantly improved the erythrocyte deformability index under 4.7 and 9.5 dyne/cm^2 shear stresses. The deformability index under 23.6 dyne/cm^2

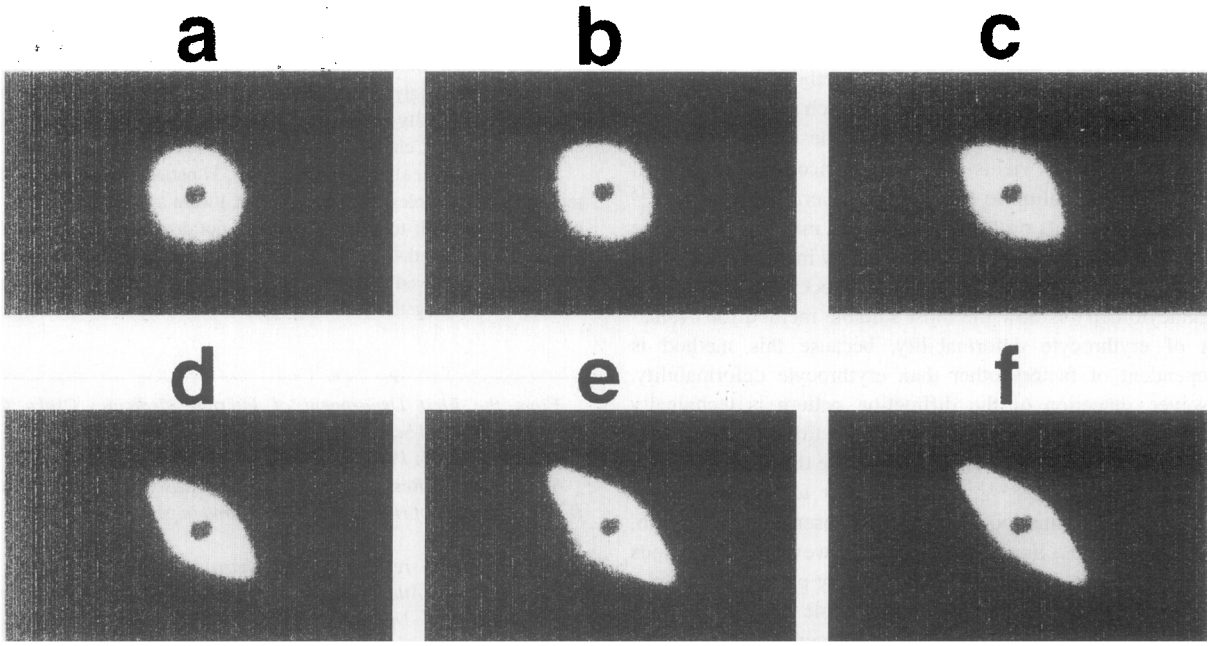

Fig 1. Diffraction patterns produced at a shear stress of 4.7 dyne/cm^2 (a), 9.5 dyne/cm^2 (b), 23.6 dyne/cm^2 (c), 47.3 dyne/cm^2 (d), 118.1 dyne/cm^2 (e), and 236.2 dyne/cm^2 (f) applied to erythrocytes of normocholesterolemic subjects.

Table 1. Clinical Characteristics and Erythrocyte Deformability Indices in Control Subjects and Hypercholesterolemic Patients and Effect of 1 Year of Pravastatin Therapy

Characteristic	Control Subjects	Hypercholesterolemic Patients	
		Pretherapy	1 Year
No. of subjects	20	37	
Age (yr)	49 ± 8	52 ± 7	
Men (%)	60	57	
Mean blood pressure (mm Hg)	97 ± 5	104 ± 13	102 ± 14
Serum creatinine (mg/dL)	0.8 ± 0.2	0.9 ± 0.3	0.9 ± 0.4
Blood urea nitrogen (mg/dL)	17 ± 2	19 ± 3	18 ± 4
Total cholesterol (mg/dL)	197 ± 12	288 ± 28*	223 ± 20‡
LDL cholesterol (mg/dL)	115 ± 13	202 ± 31*	136 ± 23‡
Triglyceride (mg/dL)	162 ± 25	186 ± 43*	181 ± 36
HDL cholesterol (mg/dL)	50 ± 7	49 ± 6	51 ± 5†
Deformability index			
4.7 dyne/cm²	494 ± 30	400 ± 54*	481 ± 37‡
9.5 dyne/cm²	987 ± 31	898 ± 76*	1,057 ± 107‡
23.6 dyne/cm²	1,984 ± 149	1,806 ± 147	1,906 ± 114
47.3 dyne/cm²	2,750 ± 185	2,520 ± 197	2,684 ± 78
118.1 dyne/cm²	3,524 ± 288	3,414 ± 179	3,591 ± 137
236.2 dyne/cm²	4,135 ± 206	4,094 ± 245	4,140 ± 168

NOTE. Values are the mean ± SD.
*$P < .05$ *v* control subjects.
†$P < .05$ *v* pretherapy.
‡$P < .01$ *v* pretherapy.

and greater tended to be increased by cholesterol-lowering therapy, but the differences were not statistically significant.

Figures 2 and 3 show the relationship of the deformability index under 4.7 dyne/cm² shear stress to total cholesterol and LDL cholesterol levels in hypercholesterolemic patients, respectively. The deformability index under this shear stress was

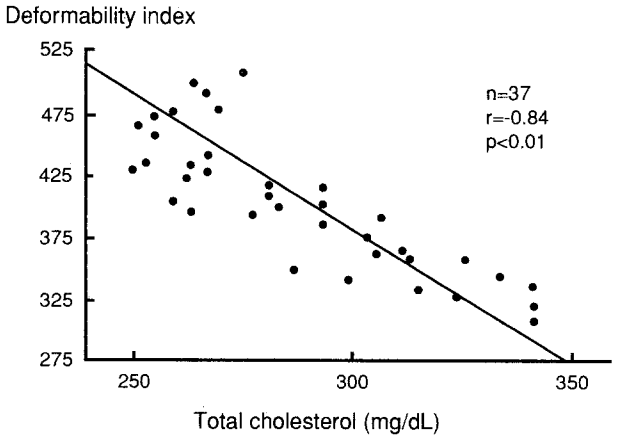

Fig 2. Relation of the erythrocyte deformability index (shear stress, 4.7 dyne/cm²) to total cholesterol level at entry in hypercholesterolemic patients.

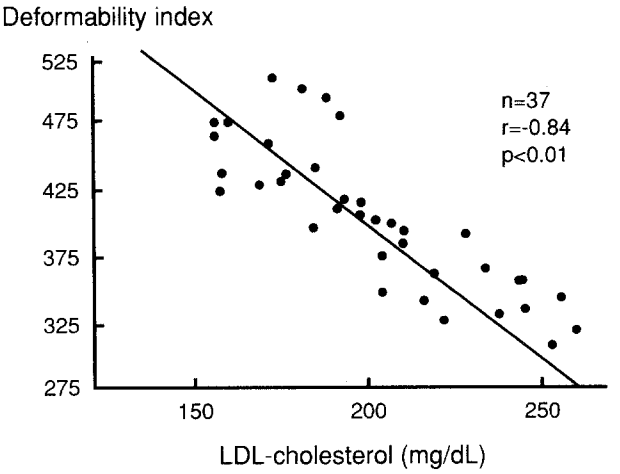

Fig 3. Relation of the erythrocyte deformability index (shear stress, 4.7 dyne/cm²) to LDL cholesterol level at entry in hypercholesterolemic patients.

inversely correlated with total cholesterol and LDL cholesterol levels. In contrast, the deformability index under this shear stress was not correlated with triglyceride and HDL cholesterol levels (N = 37, $r = .11$ and N = 37, $r = .18$, respectively). The deformability index under 9.5 dyne/cm² shear stress was also inversely correlated with total cholesterol and LDL cholesterol levels (N = 37, $r = -.62$, $P < .01$ and N = 37, $r = -.69$, $P < .01$, respectively). However, the deformability index under this shear stress was not correlated with triglyceride and HDL cholesterol levels (N = 37, $r = .08$ and N = 37, $r = .14$, respectively).

The relation between the improvement (percent change) of the deformability index under 4.7 dyne/cm² shear stress and the reduction of serum cholesterol and LDL cholesterol is shown in Figs 4 and 5, respectively. Improvement of the deformability index under 4.7 dyne/cm² shear stress was strongly correlated with the reduction of serum cholesterol and LDL cholesterol,

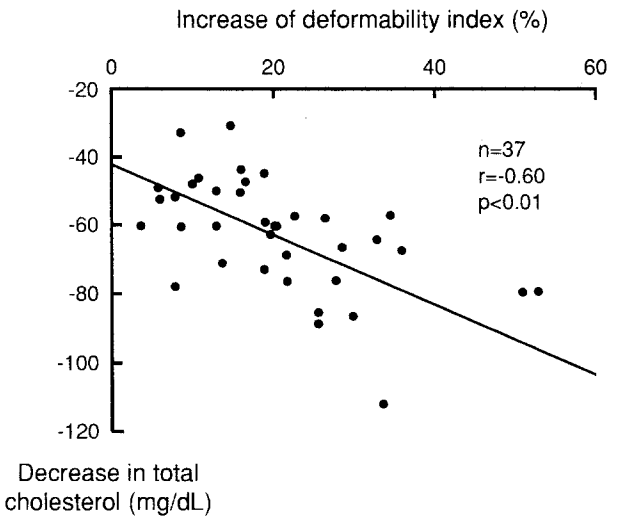

Fig 4. Relation of changes (%) in the erythrocyte deformability index (shear stress, 4.7 dyne/cm²) to decreases in total cholesterol level during a 1-year cholesterol-lowering therapy with pravastatin.

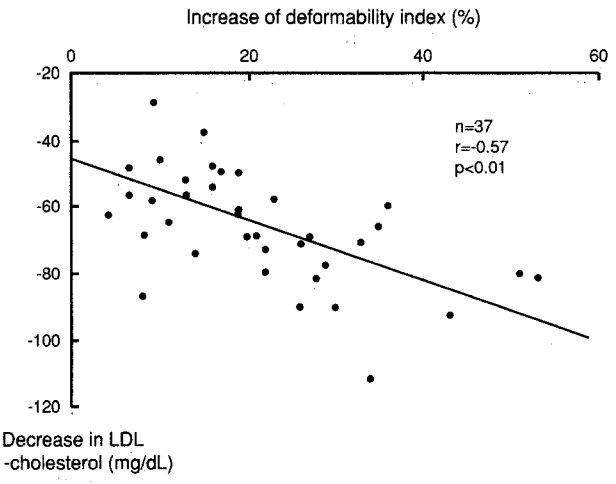

Fig 5. Relation of changes (%) in the erythrocyte deformability index (shear stress, 4.7 dyne/cm^2) to decreases in LDL cholesterol level during a 1-year cholesterol-lowering therapy with pravastatin.

respectively. On the other hand, this improvement was not related to the alteration in HDL cholesterol (N = 37, r = .17) or triglyceride (N = 37, r = −.12).

Improvement of the deformability index under 9.5 dyne/cm^2 shear stress was also significantly correlated with the reduction in serum cholesterol and LDL cholesterol (N = 37, r = −.34, P < .05 and N = 37, r = −.30, P < .05, respectively). This parameter was not related to the alteration in HDL cholesterol (N = 37, r = .11) or triglyceride (N = 37, r = −.15).

DISCUSSION

The major aim of cholesterol-lowering therapy is to reduce the risk for ischemic heart disease.[24] Ischemic heart disease is mainly caused by a disturbance in coronary artery blood flow. Coronary artery blood flow is determined by anatomical and functional factors such as blood rheology or platelet activity. Furthermore, the viscoelastic properties of the erythrocyte are important determinants of microcirculatory coronary blood flow and atherosclerosis. Actually, changes in erythrocyte deformability affect blood viscosity, and they influence coronary blood flow. Such functional factors determining coronary blood flow are found to be affected by blood cholesterol levels.[11,12] On the other hand, since hypercholesterolemia is a cause of accelerated atherosclerosis, it is reasonable to suppose that cholesterol-lowering therapy will retard the progression of coronary atherosclerosis. However, recent clinical trials have demonstrated that cholesterol-lowering therapy markedly reduces cardiovascular events associated with a modest regression of atherosclerotic stenosis.[2-4] During the cholesterol-lowering therapy, angina symptoms are decreased within months of treatment, in which time regression of coronary atherosclerosis is unlikely to occur.[2-4] These observations may raise the hypothesis that a marked reduction in clinical cardiovascular events by decreasing serum cholesterol is at least in part due to an improvement in hemorrheology such as erythrocyte deformability, blood viscosity, and platelet activation state, as well as regression of atherosclerotic stenosis per se. Actually, cholesterol-lowering therapy with lovastatin was recently shown to improve erythrocyte filterability in familial hypercholesterolemia patients.[25] However, little is known about the effect of cholesterol-lowering therapy on erythrocyte deformability as determined by ektacytometry, which is independent of factors (other than erythrocyte deformability) that influence blood viscosity, such as the hematocrit, plasma viscosity, and cell aggregation. Previously, several studies using ektacytometry have shown that erythrocyte deformability is reduced in cholesterol-fed animals.[26,27] In the present study, we demonstrated for the first time using ektacytometry that erythrocyte deformability under 4.7 and 9.5 dyne/cm^2 shear stresses in hypercholesterolemic patients is reduced by approximately 20% compared with that in age-matched normocholesterolemic subjects, and that the deformability is inversely correlated with serum cholesterol and LDL cholesterol. Furthermore, cholesterol-lowering therapy with pravastatin improved erythrocyte deformability under 4.7 and 9.5 dyne/cm^2 shear stresses, and this improvement was significantly related to the reduction in serum cholesterol and LDL cholesterol. These results suggest that reduced erythrocyte deformability in hypercholesterolemic patients is at least in part related to plasma cholesterol levels and hence is reversible with cholesterol-lowering therapy. Therefore, our observation that pravastatin treatment was associated with a significant improvement in erythrocyte deformability may be interpreted in terms of a beneficial effect on organ perfusion, including microcirculatory coronary blood flow.

The most probable explanation for the reduced erythrocyte deformability under a weak shear stress in hypercholesterolemic patients is that an increase in cholesterol content of the erythrocyte induces a reduction of erythrocyte deformability. Actually, under in vitro conditions, incubation of normal human erythrocytes in cholesterol-enriched plasma leads to an increase in the cholesterol level of the erythrocyte and to a reduction in the deformability.[28-30] In addition, the cholesterol and phospholipids constituting an integral part of the erythrocyte are exchangeable between the plasma and the erythrocyte.[31] This explanation may also be supported by our finding that the improvement of erythrocyte deformability under a weak shear stress is related to the reduction in serum cholesterol and LDL cholesterol during pravastatin therapy. On the other hand, there was no significant difference in erythrocyte deformability under a strong shear stress (≥23.6 dyne/cm^2) between hypercholesterolemic patients and normal controls. In this respect, Bull et al[22] have demonstrated that erythrocyte deformability under a weak shear stress is mainly governed by the viscoelastic properties of the cell membranes, whereas deformability under a strong shear stress is mainly governed by the ratio of cell surface area to volume (Table 2).

There are several possible mechanisms by which the increase of membrane cholesterol content reduces erythrocyte deform-

Table 2. Characteristics of Erythrocyte Deformability Measurements Made by Detection of Light-Intensity Patterns of Diffraction Images From Ektacytometry

	Weak Shear Stress (<10 dyne/cm^2)	Strong Shear Stress (>20 dyne/cm^2)
Major determinant	Viscoelastic properties of cell membrane	Ratio of cell surface area to volume
Clinical application	Hypercholesterolemia	Spherocytosis?

ability. One possible mechanism is that the increase of membrane cholesterol content increases intracellular Ca^{2+} concentration by reducing the activity of (Ca^{2+},Mg^{2+})-adenosine triphosphatase, thereby inducing the reduction of erythrocyte deformability.[29]

Another possible mechanism is that the increase of erythrocyte membrane cholesterol content results in a gross distortion of the membrane,[32] which directly influences deformability. However, the exact cellular mechanism by which the increase of erythrocyte cholesterol content reduces deformability remains to be clarified.

In conclusion, the present study using diffractometry has shown that erythrocyte deformability under a weak shear stress, which reflects viscoelastic properties of erythrocyte membranes, is reduced in hypercholesterolemic patients, and that cholesterol-lowering therapy with pravastatin improves erythrocyte deformability by a reduction in serum cholesterol and LDL cholesterol in these patients, which may contribute to the improvement in myocardial perfusion. However, further studies will be required to clarify the relation between the reduction of erythrocyte deformability and the reduction[33] of cardiovascular events by cholesterol-lowering therapy.

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