Effects of myakuryu on hemorheological characteristics and mesenteric microcirculation of rats fed with a high-fat diet

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Abstract. There is evidence that hyperlipidemia can induce hemorheological and microcirculatory disturbances. Myakuryu, a Chinese traditional medicine is efficacious in promoting lipid metabolism and protecting oxidative stress, but whether this drug can ameliorate rheologic disturbances caused by hyperlipidemia is still unknown. The present study was conducted to investigate the effects of myakuryu on hemorheological and microcirculatory disturbances induced by hyperlipidemia. Wistar rats were divided into a group on control diet (n = 8) and a group on high-fat diet (HFD, n = 44). Eight weeks later, plasma triglyceride (TG) and total cholesterol (TC) were determined. Sixteen animals with the highest levels of hyperlipidemia from the HFD group were randomly divided into two sub-groups: the untreated hyperlipidemia group (n = 8) and the group treated with myakuryu (n = 8). At the end of the sixteenth week, rheological and microcirculatory parameters were measured. Chemical analysis showed that myakuryu treatment caused significant reductions of plasma TG and TC levels (P < 0.01), and the cholesterol/phospholipid ratio in the erythrocyte membrane (P < 0.05). Rheological and microcirculatory measurements showed that myakuryu treatment led to a significant decrease in the erythrocyte aggregation index, plasma viscosity and blood viscosity at shear rates of 50, 100 and 150 s⁻¹ and in adherent leukocytes in mesenteric venules. There was a significant increase in erythrocyte deformation, electrophoretic mobility, membrane fluidity and F-actin content in the erythrocyte membrane as well as in red cell velocity in mesenteric venules. Our findings suggest that myakuryu treatment can improve blood flow and reduce adherent leukocytes in the venules of rats fed with HFD by ameliorating blood viscosity, erythrocyte deformability and aggregation, and other hemorheological characteristics.

Keywords: Myakuryu, hyperlipidemia, hemorheological characteristics

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1. Introduction

It is generally acknowledged that atherosclerosis is multifactorial in nature, and there is considerable evidence that hemodynamic hindrance is one of the important contributors to the development of atherosclerosis [9]. Many studies have revealed that hyperlipidemia can lead to rheologic disturbances, which are implicated in the pathogenesis of atherosclerosis through their effects on the microcirculation [41]. Rheological and microcirculatory parameters are also important markers for the treatment of atherosclerosis.

Myakuryu, a new type of traditional Chinese medicine mainly present in the extracts of *Crataegus oinnatifida bge* (COB), *Panax notoginseng* (PN) and *Ginkgo billoba* (GB), has been used clinically to prevent the progression of atherosclerosis in China and Japan, but its mechanisms of action are still not completely understood. According to traditional Chinese medicine theory, COB, PN and GB all have cardioprotective benefits [24]. In general, therapeutic effects of compounds in Chinese medicine are the combined results of multiple bioactive components. Pharmacological research has revealed that flavonoids, saponins and terpenoids are the main bioactive components of COB, PN and GB [14,19,21,24,33].

Recently, Han et al. demonstrated that myakuryu could improve the microcirculatory disturbance induced by ischemia-reperfusion in rats [14]. However, little is known about whether this drug can ameliorate the hemorheological and microcirculatory disturbances caused by hyperlipidemia. Our study is the first to evaluate the effects of myakuryu on hemorheological and microcirculatory disturbances in rats with hyperlipidemia and the underlying mechanisms.

2. Materials and methods

2.1. The drug

Myakuryu was provided by Tasly Pharmaceutical Co. (Tianjin, China; Patent Proposal: 200510069450.7 in China, 2004-140288 in Japan). The components are as follows [14]: 85% *Crataegus oinnatifida bge* extract (including flavonoid = 3–5%), 10% *Panax notoginseng* extract (including saponins = 30%) and 5% *Ginkgo billoba* extract (including flavonoids = 24%, terpenoid = 6%, ginkgolic acid < 5 ppm).

2.2. Animals

Wistar rats, weighing 280 ± 20 g, were supplied by the Department of Experimental Animals, Health Science Centre, Peking University, Beijing, China, and handled according to the guidelines of the Peking University Animal Research Committee. The rats were housed in a room with temperature (22 ± 2°C) and humidity (55 ± 10%) controls for seven days before experimentation. Then, the animals were randomly divided into two groups: control group (C-group, n = 8), fed with standard laboratory chow, and high-fat diet (HFD) group (n = 44), fed with high-lard/cholesterol chow, including 82.5% standard laboratory chow, 2% cholesterol, 10% lard, 5% vitellus powder and 0.5% sodium cholate. All animals were provided free access to the diet and water. Eight weeks later, blood was collected from overnight-fasted rats by retro-orbital bleeding and anti-coagulated with heparin. Plasma was isolated by centrifugation to determine the total cholesterol (TC) and triglyceride (TG) levels. Then, 16 rats with the highest plasma
lipid levels were selected from the HFD group and again randomly divided into two groups: an untreated hyperlipidemia group (H-group, \( n = 8 \)), fed for eight weeks with HFD only without myakuryu, and a group of HFD rats treated with myakuryu (M-group, \( n = 8 \)), fed for eight weeks with HFD plus myakuryu, 400 mg/kg/day, i.g.

2.3. Observation of mesenteric microcirculation

At the end of the 16th week, the rats were anesthetized with 20% ethylcarbamate at a dose of 1 ml/100 g (i.m.). The abdomen was opened by midline laparotomy. The ileocecal junction of the mesentery was gently drawn out and spread over a transparent vitric stage specially designed for the rat. The rat’s body temperature was maintained at 37°C using a homeothermic blanket system. The exposed intestine was covered with gauze continuously wetted with warm saline solution (37°C). Microcirculatory hemodynamics in the mesentery was observed by a transillumination method using an inverted microscope (DM-IRB, Leica, Germany) fixed into the homeothermic blanket. Images of the venules were recorded by a CCD video camera system (JK-TU53H, Toshiba, Japan).

The diameters of venules were measured using an Image-Pro Plus 5.0 software (Media Cybernetics, USA). The diameter at each location was taken from the mean of three measurements. Single unbranched venules without an obvious bend and with diameters ranging from 25 to 35 µm and lengths of about 200 µm were randomly selected from the recorded videotape images to determine the number of adherent leukocytes and the RBC velocity. The dynamic behavior of leukocytes was reviewed by replaying the recorded video images. Adherent leukocytes were defined as cells that attached to the same site for more than 30 s. The number of adherent leukocytes was counted along each venule over a length of 200 µm. The velocity of RBC in the venules was recorded for 10 s at a rate of 2000 frames/s by using a high-speed video camera system (FASTCAM-ultra APX, Photron, Japan), and the stored high-speed images were replayed at a rate of 25 frames/s. RBC velocity in the venules was measured using the Image-Pro Plus 5.0 software.

2.4. Blood treatment and plasma lipid analysis

After observing the microcirculation, blood was drawn by abdominal aorta puncture and anticoagulated with heparin. The anti-coagulated blood was divided into two samples: one (1.5 ml) was directly taken to measure hematologic parameters and whole blood viscosity, and the other (5–7 ml) was centrifuged at 1000 rpm for 5 min. After centrifugation, the plasma was aspirated for plasma lipid analysis and plasma viscosity measurement; theuffy coat was also removed by aspiration. The remaining RBC were then washed three times with isotonic phosphate-buffered saline (PBS) solution and used for other assays. Plasma TG and TC levels were analyzed enzymatically using commercial kits (Sigma, WI, USA).

2.5. Measurement of the ratio of cholesterol and phospholipids in erythrocyte membranes

Erythrocyte membranes were prepared according to Dodge et al. [11]. Lipids were extracted from membranes by using a chloroform/isopropanol mixture (1/1 (v/v)) [29]. The cholesterol (Ch) concentration was determined based on the protocol of Warnick [36]; and the phospholipid (Pl) concentration was determined by analyzing the phosphorous content in the extracts, as described by Bartlett [2]; then the Ch/Pl ratio was calculated.
2.6. Measurement of hematological parameters of erythrocytes

Twenty microliters of fresh blood was collected and applied to a fully automated hematological analyzer (Sysmex KX-21N, Japan) for determinations of blood cell counts, hematocrit (HCT), mean corpuscular volume (MV), hemoglobin (HGB) and mean corpuscular hemoglobin (MCH).

2.7. Measurement of rheologic indexes

The washed RBC were suspended in 15% polyvinylpyrrolidone (PVP, MW 30 kDa) buffer (61 mM NaCl, 0.8 mM Na2HPO4, 0.2 mM KH2PO4, pH 7.4, 290 mOsm/kg, viscosity 15 mPa-s) and adjusted to $2 \times 10^7$ cells/ml to measure the deformation index (DI) by using an ektacytometer (Model LBY-BX2, Precil Company, China) at a series of shear rates from 50 to 1000 s$^{-1}$ [37]. The value of DI at the maximum shear rate of 1000 s$^{-1}$ is designated as $(DI)_{max}$, and integrated deformation index (IDI) is the integral of DI at the shear rates of 50–1000 s$^{-1}$. In our low-shear ektacytometry, RBC were suspended in 3% PVP buffer (15% PVP/PBS: 1/4) and adjusted to $2 \times 10^7$ cells/ml. The orientation index (DI)$_{or}$ was measured at a series of shear rates from 50 to 150 s$^{-1}$ [38,40]. Whole blood viscosity was measured by using a cone–plate viscometer (Model LBY-N6A, Precil Company, China) at shear rates $\dot{\gamma} = 50, 100$ and 150 s$^{-1}$. Plasma viscosity was measured with a capillary viscometer (Model LBY-NM2, Precil Company, China). Erythrocyte aggregation index (AI) was determined in the ektacytometer by monitoring the rate of change in the optical reading when the sample was brought to a standstill after pre-shearing (to cause total disaggregation) at $\dot{\gamma} = 600$ s$^{-1}$. All the rheologic measurements were carried out at 37°C according to the International Guidelines for the measurements of rheologic parameters [15].

2.8. Measurement of RBC electrophoretic mobility

The washed RBC were adjusted to $2 \times 10^6$ cells/ml with 0.9% (W/V) sodium chloride. The electrophoretic mobility (EPM) was measured (voltage 40 V, 30°C) by using a Cell Electrophoresis Apparatus (LIANG-100, Shanghai Medical University, China). The mean value of measurements on ten cells was determined for each blood sample.

2.9. Measurement of RBC membrane fluidity

Membrane fluidity was studied by determining the fluorescent polarization parameter $p$ and membrane microviscosity $\eta$. The washed RBC ($2 \times 10^6$ cells/ml) in PBS were mixed with an equal volume of $2 \times 10^{-6}$ M 1,6-diphenyl-1,3,5-hexatriene (DPH) (Fluka Chemie AG, Switzerland), dissolved in tetrahydrofuran, and incubated at 37°C for 30 min. The fluorescence polarization was determined by using a spectrophotofluorimeter (Hitachi, Japan). The excitation and emission wavelengths were 360 and 430 nm, respectively. The fluorescence polarization parameter $p$ was determined according to the formula: $p = (I_{VV} - GI_{VH})/(I_{VV} + GI_{VH})$, where $I_{VV}$ is the fluorescent intensity when the light axis of the polarizer and the analyzer are both in the vertical direction; $I_{VH}$ is the fluorescent intensity when the light axis of polarization is in the vertical direction, whereas that of the analyzer is in the horizontal direction; $G$ is the correction factor. $G = I_{HV}/I_{HH}$, where $I_{HH}$ is the fluorescent intensity when the light axis of the polarizer and the analyzer are both in the horizontal direction; $I_{HV}$ is the fluorescent intensity when the light axis of the polarizer is in the horizontal direction, whereas that of the analyzer is in the vertical direction [13]. Membrane microviscosity $\eta$ was calculated according to the formula: $\eta = 2p/(0.46 - p)$. The values of $p$ and $\eta$ are inversely related to the membrane fluidity.
2.10. Confocal laser scanning microscopy analysis

The washed RBC (2 × 10^6 cells/ml) were fixed in 3.7% formaldehyde solution in PBS for 10 min at room temperature. After washing twice in PBS, RBC were re-suspended in 0.1% Triton X-100/PBS for 5 min at room temperature and then incubated in 1% BSA/PBS for 30 min to reduce the nonspecific staining. After washing in PBS, RBC were labeled with 2U of a fluorescent derivative of phalloidin (Rhodamine phalloidin, Molecular Probes, Eugene, OR) for 20 min in the dark. The cells were washed with PBS once and re-suspended in 50 µl of PBS. A small chamber was positioned on the glass slide with the use of double-faced adhesive tape. The suspension was dropped into the chamber, which was covered with a cover glass and mounted on the stage of the confocal laser scanning microscopy (CLSM) (Leica Laser-technik, Germany). A 568-nm laser was used for the excitation of rhodamine phalloidin. A 580-nm long-pass filter was placed in the fluorescence detection path. The mean relative fluorescence intensities were determined by using the Leica software function.

2.11. Statistical analysis

All results were presented as means ± standard deviations (SD). The statistical analysis was performed with SPSS 11.5 software. Differences between groups were analyzed by ANOVA. The condition of \( P < 0.05 \) was considered to be statistically significant.

3. Results

3.1. Body weight, plasma lipid levels and the Ch/Pl ratio of erythrocyte membranes

The body weight (BW) of the rats in the H-group was higher than that in the C-group (\( P < 0.05 \)). No statistically significant difference in BW was observed between the M- and H-groups (Table 1).

Plasma lipid analysis showed that, after 16 weeks of high-fat diet, the H-group showed increased TG and TC levels compared to the C-group (\( P < 0.01 \)), whereas, after myakuryu treatment for 8 weeks, the M-group showed significantly lower TG and TC levels compared to the H-group (\( P < 0.01 \); Table 1).

Membrane Ch/Pl ratio is one of the main factors that determine the biophysical properties of the erythrocyte membrane, and thus erythrocyte rheology. The results showed that the Ch/Pl ratio of erythrocyte membranes increased significantly in the H-group compared to the C-group (\( P < 0.05 \)). After myakuryu treatment for 8 weeks, the ratio decreased significantly (\( P < 0.05 \)) in the M-group compared to the C-group (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>TG (mmol/l)</th>
<th>TC (mmol/l)</th>
<th>Membrane Ch/Pl</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-group</td>
<td>402 ± 64</td>
<td>0.65 ± 0.07</td>
<td>1.49 ± 0.12</td>
<td>0.795 ± 0.028</td>
</tr>
<tr>
<td>H-group</td>
<td>557 ± 128</td>
<td>1.04 ± 0.15</td>
<td>2.32 ± 0.31</td>
<td>0.915 ± 0.041</td>
</tr>
<tr>
<td>M-group</td>
<td>497 ± 91</td>
<td>0.75 ± 0.15</td>
<td>1.82 ± 0.21</td>
<td>0.837 ± 0.053</td>
</tr>
</tbody>
</table>

Compared with C-group, \(^a P < 0.05, \(^b P < 0.01\); compared with H-group, \(^c P < 0.05, \(^d P < 0.01\).
3.2. Hematologic and rheologic parameters

Erythrocyte counts, HCT, MCV, HGB and MCH measurements did not show significant differences among the three groups ($P > 0.05$; Table 2).

The erythrocyte deformation index DI reflects the combination of membrane flexibility and cytoplasmic viscosity [39], while the orientation index $(\text{DI})_{or}$ and integrated deformation index IDI are signs of the morphology of erythrocytes and membrane deformation in the field of flow [38]. The results showed that $(\text{DI})_{max}$, $(\text{DI})_{or}$ and IDI significantly decreased in the H-group ($(\text{DI})_{max} P < 0.01$; IDI and $(\text{DI})_{or} P < 0.05$); and that $(\text{DI})_{max}$ and IDI increased significantly in the M-group ($(\text{DI})_{max} P < 0.01$; IDI $P < 0.05$) after myakuryu treatment (Table 3). However, no statistically significant difference in $(\text{DI})_{or}$ was observed between M- and H-groups (Table 3).

Whole blood and plasma viscosity are major indicators of blood flow properties. The results showed that the whole blood viscosity ($\dot{\gamma} = 50, 100, 150$ s$^{-1}$; $P < 0.05$) and plasma viscosity in the H-group were higher than those in the C-group ($P < 0.01$). After myakuryu treatment, blood viscosity ($\dot{\gamma} = 50, 100$ s$^{-1}$; $P < 0.01$; $\dot{\gamma} = 150$ s$^{-1}$; $P < 0.05$) and plasma viscosity ($P < 0.05$) were significantly lower in the M- than in the H-group (Table 3).

3.3. Electrophoretic mobility (EPM) and aggregation index (AI) of erythrocytes

The results showed that EPM decreased significantly in the H-group ($P < 0.01$), indicating a lower surface charge density in erythrocytes of rats with hyperlipidemia (Table 4). The diminished surface charge density would result in decreased repulsion of erythrocytes, hence increasing erythrocyte aggregation [25]. The results showed that the erythrocyte AI in the H-group was higher than that in the C-group ($P < 0.01$). After myakuryu treatment for 8 weeks, EPM increased and the AI decreased significantly in the M-group as compared to the H-group ($P < 0.01$; Table 4).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>HCT (l/l)</th>
<th>Counts ($\times 10^{12}$ cells/l)</th>
<th>MCV (fl)</th>
<th>HGB (g/l)</th>
<th>MCH (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-group</td>
<td>0.439 ± 0.019</td>
<td>8.94 ± 0.94</td>
<td>55.84 ± 2.65</td>
<td>159.38 ± 13.31</td>
<td>21.15 ± 1.58</td>
</tr>
<tr>
<td>H-group</td>
<td>0.437 ± 0.024</td>
<td>8.78 ± 0.68</td>
<td>55.50 ± 3.06</td>
<td>156.63 ± 15.30</td>
<td>20.89 ± 2.00</td>
</tr>
<tr>
<td>M-group</td>
<td>0.446 ± 0.035</td>
<td>8.98 ± 1.54</td>
<td>55.16 ± 3.25</td>
<td>156.88 ± 22.04</td>
<td>20.91 ± 1.44</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>$(\text{DI})_{max}$ (%)</th>
<th>IDI (%)</th>
<th>$(\text{DI})_{or}$ (%)</th>
<th>Plasma viscosity (mPa·s)</th>
<th>Whole blood viscosity (mPa·s)</th>
<th>$\dot{\gamma} = 50$ s$^{-1}$</th>
<th>$\dot{\gamma} = 100$ s$^{-1}$</th>
<th>$\dot{\gamma} = 150$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-group</td>
<td>50.88 ± 0.92</td>
<td>38.18 ± 1.17</td>
<td>31.53 ± 1.57</td>
<td>1.52 ± 0.12</td>
<td>5.11 ± 0.29</td>
<td>4.39 ± 0.25</td>
<td>3.91 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>H-group</td>
<td>46.81 ± 1.62$^b$</td>
<td>34.45 ± 1.27$^a$</td>
<td>29.81 ± 1.47$^a$</td>
<td>1.83 ± 0.13$^b$</td>
<td>5.89 ± 0.30$^b$</td>
<td>5.08 ± 0.29$^b$</td>
<td>4.47 ± 0.32$^b$</td>
<td></td>
</tr>
<tr>
<td>M-group</td>
<td>49.38 ± 1.41$^d$</td>
<td>36.25 ± 1.35$^c$</td>
<td>30.63 ± 1.48</td>
<td>1.67 ± 0.12$^c$</td>
<td>5.16 ± 0.36$^d$</td>
<td>4.59 ± 0.32$^d$</td>
<td>4.03 ± 0.31$^c$</td>
<td></td>
</tr>
</tbody>
</table>

Compared with C-group, $^aP < 0.05$, $^bP < 0.01$; compared with H-group, $^cP < 0.05$, $^dP < 0.01$. 


Table 4

<table>
<thead>
<tr>
<th></th>
<th>EPM (µm/s/V/cm)</th>
<th>AI (%)</th>
<th>Fluorescence polarization, p</th>
<th>Microviscosity, η (mPa·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-group</td>
<td>0.950 ± 0.027</td>
<td>48.70 ± 1.85</td>
<td>0.175 ± 0.014</td>
<td>1.193 ± 0.215</td>
</tr>
<tr>
<td>H-group</td>
<td>0.833 ± 0.026&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.78 ± 2.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.201 ± 0.020&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.574 ± 0.279&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M-group</td>
<td>0.977 ± 0.042&lt;sup&gt;d&lt;/sup&gt;</td>
<td>48.30 ± 1.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.181 ± 0.018&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.283 ± 0.264&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Compared with C-group, <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01; compared with H-group, <sup>c</sup>P < 0.05, <sup>d</sup>P < 0.01.

3.4. Membrane fluidity of erythrocytes

The fluorescence polarization parameter, p, and microviscosity, η, were significantly higher in the H-group than in the C-group (P < 0.05). After myakuryu treatment, both p and η decreased significantly in the M-group as compared to the H-group (P < 0.05), reflecting an increased membrane fluidity (Table 4).

3.5. Mean fluorescent intensity of F-actin under erythrocyte membrane

F-actin was studied with CLSM to determine the mean fluorescent intensity, which reflects its relative content under the membrane. The results showed that the fluorescent intensity in the H-group was significantly lower than that in the C-group (P < 0.01). Treatment with myakuryu for 8 weeks resulted in a significant increase in fluorescent intensity in M- as compared to the H-group (P < 0.01), suggesting that the content of F-actin in the erythrocyte submembrane increased after myakuryu treatment (Figs 1 and 2).

3.6. RBC velocity and leukocyte adhesion in mesenteric venules

No significant differences in the diameter of mesenteric venules were detected among the rats of the three groups (Table 5). Image analysis showed that the RBC velocity in the mesenteric venules of the rats in the H-group was significantly lower than that in the C-group (P < 0.05). After myakuryu treatment for 8 weeks, the RBC velocity of rats in the M-group was higher than that in the H-group (P < 0.05; Table 5).

Live video images revealed that many leukocytes appeared in the venules rolling along the venular wall in the rats of the H-group, and some of them were observed to adhere to the venular wall and form aggregates. Image analysis showed that, after 16 weeks of high-fat diet, the H-group showed significantly increased adherence of leukocytes in the mesenteric venules compared to the C-group (P < 0.01), whereas, after myakuryu treatment for 8 weeks, the M-group showed significantly lower leukocyte adherence compared to the H-group (P < 0.05; Fig. 3 and Table 5).

4. Discussion

In this study, we treated the HFD-induced hyperlipidemic rats with myakuryu and investigated its effects on blood cell rheology and on flow and cell adhesion in the microcirculation. The results showed that plasma TG and TC levels increased significantly in the rats fed with HFD alone. Myakuryu treatment resulted in a significant reduction of TG and TC levels in the hyperlipidemic rats induced by HFD. These results demonstrate that myakuryu is effective in lowering plasma lipid. The bioactive components of
Fig. 1. Images of F-actin beneath erythrocyte membrane (magnification: 400×). C: C-group; H: H-group; M: M-group.

Fig. 2. Fluorescent intensity of F-actin in the three groups. The intensity values were normalized using the mean value of the H-group as 100%. * \( P < 0.01 \), compared with C-group. # \( P < 0.01 \), compared with H-group.

Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter (µm)</th>
<th>RBC velocity (mm/s)</th>
<th>Adherent leukocytes (No. of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-group</td>
<td>31.53 ± 2.57</td>
<td>1.24 ± 0.13</td>
<td>4.03 ± 0.18</td>
</tr>
<tr>
<td>H-group</td>
<td>30.18 ± 2.71</td>
<td>0.91 ± 0.12</td>
<td>14.75 ± 1.04</td>
</tr>
<tr>
<td>M-group</td>
<td>30.50 ± 1.97</td>
<td>1.28 ± 0.24</td>
<td>10.73 ± 1.97</td>
</tr>
</tbody>
</table>

Compared with C-group, \( ^a P < 0.05 \), \( ^b P < 0.01 \); compared with H-group, \( ^c P < 0.05 \).

myakuryu may play an important role for its lipid-lowering actions. It has been reported that flavonoids, saponins and terpenoids can activate the peroxisome proliferator-activated receptors (PPARs) [16,34]. The activated PPARs (mainly PPAR\( _\alpha \) and PPAR\( _\gamma \)) can promote lipid metabolism through regulating the expression of the enzymes in lipid metabolism [19]. The elevated plasma lipid can create problems in
blood flow [35]. Our results showed that RBC velocity in the mesenteric venules of the HFD rats was markedly lower than in the control rats on a normal diet. The lower blood flow rate, RBC aggregation, and the interaction between erythrocytes and leukocytes may cause leukocytes to depart from axial flow and marginate towards the venular wall [5,8,12,26]. In our experiments, the number of adherent leukocytes increased markedly in the mesenteric venules of the HFD rats. After myakuryu treatment, RBC velocity increased and adherent leukocytes decreased significantly. Thus, amelioration of rheologic disturbances may be an important factor for myakuryu to improve RBC velocity in the microcirculation. At a given vessel diameter, blood viscosity, erythrocyte aggregation, and erythrocyte deformability are important variables in determining RBC velocity. In our experiment, the diameters of the venules, which were selected randomly to measure RBC velocity, showed no significant differences among the three groups.

Blood viscosity reflects the intrinsic resistance to flow within vessels. There is evidence that blood viscosity is elevated in patients with hyperlipidemia [32]. High blood viscosity retards the flow rate and results in stasis and occlusion, which may increase the propensity for atherosclerosis [30]. In the current study, the whole blood viscosity at shear rates of 50, 100 and 150 s$^{-1}$ and plasma viscosity were elevated in the HFD rats, and the viscosities decreased markedly after myakuryu treatment. This may be one of the reasons that myakuryu increases RBC velocity. The major determinants of blood viscosity are hematocrit, plasma viscosity, erythrocyte aggregation and deformability [6]. In our experiments, erythrocyte counts and hematocrit measurements did not show significant differences among three groups; the reduction of plasma viscosity and the amelioration of erythrocyte aggregation and deformability can also contribute to the decreased blood viscosity following myakuryu treatment.

Erythrocyte deformability is one of the important variables that influence blood rheological behavior. Decreased erythrocyte deformability could directly impair RBC velocity. At a high shear stress, erythrocyte deformability is also an important determinant of whole blood viscosity. In the present study, $(DI)_{\text{max}}$, IDI and $(DI)_{\text{or}}$ all decreased in the HFD rats. Myakuryu treatment caused marked increases in $(DI)_{\text{max}}$ and IDI, but no statistically significant changes in $(DI)_{\text{or}}$. These results suggest that myakuryu is capable of improving erythrocyte membrane flexibility and membrane deformation, but does not change the erythrocyte morphology in the field of flow.

Erythrocyte deformability is mainly determined by membrane viscoelasticity that is controlled by the biochemical components and a network of cytoskeletal proteins in the membrane, as well as their interactions [7]. The erythrocyte membrane is mostly composed of a lipid bilayer, whose main components are cholesterol and phospholipid. Cholesterol, especially the $Ch/Pl$ ratio, plays a key role in determining

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**Fig. 3.** Video images show leukocyte adhesion in mesenteric venules of three groups (magnification: 400×). Arrows show the adherent leukocytes. C: C-group, there is no adherent leukocyte in this group. H: H-group, there are a large number of adherent leukocytes in this group, some of them have aggregated and formed a cell clump. M: M-group, there are fewer adherent leukocytes in this group.
membrane fluidity and thus erythrocyte deformability. Because the erythrocyte has no fatty acid biosynthetic ability, its membrane lipid is largely independent of the circulating plasma lipids. It has been demonstrated that hyperlipidemia can lead to the enrichment of cholesterol in erythrocytes membrane, and a high Ch/Pl ratio [23]. Consistent with these findings, our data showed that the Ch/Pl ratio of erythrocyte membranes in the HFD rats was significantly higher than that in control group; and myakuryu treatment resulted in a marked decrease in the ratio. This may be the result of the lipid-lowering effects of myakuryu.

Membrane fluidity is a basal parameter that reflects membrane dynamics and, in turn, cell deformability. In this study, erythrocyte membrane fluidity was studied by determining the fluorescent polarization parameter \( p \) and membrane microviscosity \( \eta \). The results showed that the values of \( p \) and \( \eta \) markedly increased in the HFD rats, suggesting lower membrane fluidity in the hyperlipidemic environment. After myakuryu treatment, \( p \) and \( \eta \) all decreased significantly. Probably, this is also the result of the lipid-lowering effects of myakuryu and its effect on the Ch/Pl ratio. Schick et al. reported that erythrocyte membrane fluidity was very sensitive to changes of cholesterol concentration in the extracellular environment [31]. Other studies, however, suggested that membrane fluidity showed a better correlation with plasma triglyceride than plasma cholesterol, and it increased in a hypertriglyceridemic environment [10,18]. These authors reported that the Ch/Pl ratio had no significant relationship to plasma total cholesterol concentration, but was inversely proportional to the plasma triglyceride concentration.

The cytoskeleton system in the membrane also plays a crucial role in erythrocyte deformability. In the cytoskeleton system, F-actin constitutes the central nodes for crosslinking by spectrin and forms a hexagonal actin–spectrin network which is the biomechanical basis of the erythrocyte membrane [26]. It has been reported that oxidative stress could make F-actin depolymerize into G-actin and that F-actin losses would cause the actin–spectrin network perturbations and lead to loss of cell surface area and deformability [17,28]. Kalfa et al. reported that the loss of F-actin beneath erythrocyte membrane could lower (DD)max by an average of 81% [17]. In addition, F-actin is primarily stabilized by an interaction of protein 3 and membrane lipid [27], and with the alteration of membrane lipid in a hyperlipidemic environment, F-actin association may be less stable. In our experiments, the F-actin distribution under the plasma membrane was studied by using CLSM. The results showed that the mean fluorescent intensity under the erythrocyte membrane decreased markedly in the HFD rats, suggesting a lower content of F-actin, but it underwent a significant increase after myakuryu treatment. This result may be partially attributed to myakuryu’s effect on membrane lipid. In addition, the anti-oxidative activities of myakuryu may play an important role in this process. There are several studies to indicate that COB, PN and GB have anti-oxidative activities [1,19,24,33]. Their bioactive, naturally occurring polyphenolic compounds, flavonoids, saponins and terpenoids, can participate in proton transfer through the hydroxyls in the benzene rings, thus directly inhibiting the production of alkoxy and hydroperoxy radicals and effectively scavenging free radicals and suppressing lipid peroxidation [1,16,19,34]. Myakuryu protected the erythrocyte membrane from oxidative damage and maintained stability of the actin–spectrin network.

Erythrocyte aggregation (as determined by the aggregation index, AI), blood flow rate (as determined by RBC velocity), and leukocyte adherence are closely associated. Our data showed that AI in the HFD rats increased significantly from that of the controls. An elevated aggregation due to high plasma lipid concentrations increased blood viscosity, thereby increasing the intrinsic resistance to flow and decreasing blood flow and shear rate. A decreased flow rate (as measured by a decreased RBC velocity) in rat mesenteric venules has been shown to increase the number of adherent and rolling leukocytes [26]. Under normal flow conditions, leukocytes tend to remain in the axial flow through small blood vessels. However, at low shear rates, the formation of erythrocyte aggregates displaces leukocytes to the periph-
ery and they become marginated [8]. Myakuryu treatment resulted in a decrease in erythrocyte aggregation, which might partly account for the increase of blood flow rate and decrease of leukocyte adherence.

Erythrocyte aggregability is affected by the glycocalyx on the membrane, in particular the presence of sialic acid residues and their distribution, because sialic acid is the charged group primarily responsible for the negative surface charge of erythrocyte [3,4]. In the current study, EPM decreased significantly in HFD rats, and increased markedly after myakuryu treatment. These results suggest that surface charges on the erythrocyte membrane are recovered after myakuryu treatment. These results may be related to the anti-oxidative activities of myakuryu that prevent oxidative damage of membrane proteins and sialic acid loss. Apart from anti-oxidative activities, the lipid-lowering effects of myakuryu may also play a role in this process. Recently, Lindbohm et al. have reported that the sialic acid content in lipoproteins is inversely proportional to the cholesterol and triglyceride concentrations [22]. In addition, it has also been reported that the reduction of \( \text{Ch} / \text{Pl} \) decreases erythrocyte aggregation [23].

In conclusion, our data demonstrate that hyperlipidemia induced by high fat diet in rats can lead to the abnormal hemorheological and microcirculatory behaviors. Treatment with myakuryu is capable of ameliorating the hyperlipidemia-induced abnormalities in hemorheological properties and microcirculation flow. The lipid-lowering and anti-oxidative effects of myakuryu may play an important role in its beneficial action in hyperlipidemic rats. The current findings have elucidated the atheroprotective effects of myakuryu by considering its actions on hemorheological characteristics and the microcirculation.

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