Brazilian Journal of Medical and Biological Research (2013) 46: 138-147, http://dx.doi.org/10.1590/1414-431X20122378
ISSN 1414-431X

Exogenous normal lymph alleviates microcirculation disturbances and abnormal hemorheological properties in rats with disseminated intravascular coagulation

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Abstract

Disturbances of the microcirculation and abnormal hemorheological properties are important factors that play an important role in disseminated intravascular coagulation (DIC) and result in organ dysfunction or failure. In the present study, we established an animal model of DIC using intravenous Dextran 500 in rats, and used exogenous normal lymph corresponding to 1/15 of whole blood volume for injection through the left jugular vein. We found that normal lymph could improve the blood pressure and survival time of rats with DIC. The results regarding the mesenteric microcirculation showed that the abnormality of the diameter of mesenteric microvessels and micro-blood flow speed in the DIC+lymph group was significantly less than in the DIC+saline group. Whole blood viscosity, relative viscosity, plasma viscosity, hematocrit (Hct), erythrocyte sedimentation rate (ESR), and electrophoresis time of erythrocytes were significantly increased in the DIC+saline group compared to the control group. The electrophoretic length and migration of erythrocytes from the DIC+saline and DIC+lymph groups were significantly slower than the control group. Blood relative viscosity, Hct, ESR, and electrophoretic time of erythrocytes were significantly increased in the DIC+lymph group compared to the control group. Whole blood viscosity, relative viscosity and reduced viscosity were significantly lower in the DIC+lymph group than in the DIC+saline group, and erythrocyte deformability index was also significantly higher than in the DIC+saline and control groups. These results suggest that exogenous normal lymph could markedly improve the acute microcirculation disturbance and the abnormal hemorheological properties in rats with DIC induced by Dextran 500.

Key words: Disseminated intravascular coagulation; Lymph; Mesenteric microcirculation; Hemorheology; Dextran 500

Introduction

The lymphatic circulation participates in tissue fluid flow and plays an important role in maintaining blood volume and homeostasis (1). Studies from our laboratory have suggested that exogenous normal lymph has a therapeutic effect on rats subjected to severe hemorrhagic shock and could reduce the disturbance in the mesenteric microcirculation. The effect of lymph is significantly greater than the same volume of normal saline as well as albumin solution (2). The anti-shock effect of lymph may not be due to restoration of blood volume or colloid osmotic pressure, and its mechanism may be related to the characteristic of low viscosity and high fatty content of lymph (3). Microcirculation disturbances and abnormal hemorheological properties are important factors in disseminated intravascular coagulation (DIC), resulting in organ dysfunction or failure (4). In the present study, we established a DIC model in the rat, and examined the effects of exogenous normal lymph on the mesenteric microcirculation and hemorheological indices.

Material and Methods

Animals

Healthy specific pathogen-free (SPF) Wistar rats (supplied by the Chinese Academy of Medical Sciences Animal Breeding Center) weighing 240-300 g were used

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Received May 20, 2012. Accepted October 4, 2012. First published online February 1, 2013.

in this study. Animals were maintained under barrier-sustained conditions (25 ± 2℃, 12:12-h light-dark cycle) and were given free access to standard laboratory chow and tap water. The research protocol was carried out after a minimal acclimation period of 5 days, complied with the Guideline for the Care and Use of Laboratory Animals (NIH Publication, 1996), and was approved by the Institutional Animal Use and Care Committee of Hebei North University. Rats were fasted for 12 h before the experiment, but allowed free access to water. The animals were divided into three groups: control group (n = 10), DIC+saline group (n = 20) and DIC+lymph group (n = 20). Half the rats (n = 10 per group) in the DIC+saline and DIC+lymph groups were used for the microcirculation observation and the other half were used for the examination of hemorheological indices. The DIC model was established by injection of Dextran 500 in the DIC+saline and DIC+lymph groups.

Preparation of exogenous normal lymph

After intravenous anesthesia with 25 mg/kg pentobarbital sodium, a midline laparotomy was performed in healthy mongrel dogs and mesenteric lymph was collected continuously into heparin-wetted sterile test tubes (5). Briefly, the bowel was eviscerated and rotated to the left. The mesenteric duct and accessory lymphatic ducts (located adjacent to the superior mesenteric artery) were then isolated from the surrounding structures using blunt dissection. The main lymphatic duct was cannulated with an in-house built metal lymphatic catheter. Lymph samples were centrifuged for 15 min at 315 *g* to remove all cell components and stored at -80℃ until tested (2).

DIC model

All rats were anesthetized with an intramuscular injection of 2% pentobarbital sodium (50 mg/kg) after preliminary anesthesia with diethyl ether and placed in the supine position. The left jugular vein and the right carotid artery were aseptically separated from the surrounding tissues and cannulated with a microcatheter for transfusion and drug administration. After intravenous heparinization and stabilization for 15 min, 10% Dextran 500 (10 mL/kg, Pharmacia Biotech, Sweden) was injected through the left jugular vein over a period of 3 min using an infusion pump (ZCZ-50, Zhejiang, China) to establish the DIC model in the DIC+saline and DIC+lymph groups (6).

After 6 min, rats in the DIC+lymph group received an amount of exogenous normal lymph corresponding to 1/15 whole blood volume, which was calculated on the basis of 1/13 body weight and diluted with 1:1 saline. Rats in the DIC+saline group only received an injection of the same amount of saline. The transfusion was carried out at a rate of 0.5 mL/min for 9～12 min. The rats in the control group were anesthetized and operated as described above, but injected only with saline.

Observation and recording of the mesenteric microcirculation

Before establishment of the DIC model, the left common carotid artery was cannulated for continuous mean arterial pressure (MAP) recording throughout the experiment with a biological signal acquisition system in the rats of both DIC+lymph and DIC+saline groups (n = 10 per group). The mesentery of the ileum hypomere was placed under the observation window of a microcirculation microscope and perfused with Krebs-Hanseleit solution using a microscale peristaltic pump (36-38℃, 10-15 drops/min). The mesenteric microcirculation was continuously observed at 400X magnification using an intravital microscope with a TV recorder equipped with a cold light source and fiber-optic transillumination. Video analysis was performed to observe changes in caliber, flow state and erythrocyte aggregation of first-order arterioles (A_1), second-order arterioles (A_2), first-order venules (V_1) and second-order venules (V_2) of the mesenteric microangium. The line flow or line-granular flow, granular-line flow, granular flow, slow granular flow, vibratory granular flow, skimming or blood sludge, stopped-flow or microvessel disappearance were judged according to blood flow state. The erythrocyte aggregation status was divided into three levels: mild, moderate, and severe. The changes in flow state and erythrocyte aggregation were analyzed semiquantitatively using the Tian Niu weighted integral standard of microcirculation (Table 1). After observation of the microcirculation, the laparotomy incision was sutured and the survival time (from Dextran injection to death) was recorded for the two groups.

Determination and assay of the hemorheological indices

After normal lymph administration or vehicle treatment for 40 min, blood samples were collected from the

Table 1. Multiple score values of blood microcirculation parameters.

	Score
Flow condition	
Line flow or line-granular flow	0
Granular-line flow	0.4
Granular flow	0.8
Slow granular flow	1.6
Vibratory granular flow	4.0
Skimming or blood sludge	5.0
Stopped-flow or microvessel disappearance	6.0
Erythrocyte aggregation	
Mild	0.2
Moderate	1.0
Severe	3.0

For the evaluation of blood microcirculation from flow condition and erythrocyte aggregation, the scores range from 0 to 9. The higher score indicates worse microcirculation.

common carotid artery of the control, DIC+saline and DIC+lymph groups (n = 10 per group) and heparinized for further assay.

Two milliliters of heparinized arterial whole blood samples was loaded to the sensing slot through a straw under negative pressure conditions, and whole blood viscosity at different shear rates from 1 to 300/s was analyzed by a comprehensive sensing method using a microcirculation, hemorheology and erythrocyte deformation integrated analyzer (ChenDu Maisai Company, 3-9D, China). The plasma sample was prepared by centrifugation at 850 *g* for 10 min after the measurement of whole blood viscosity, and plasma viscosity was analyzed using the methods described above.

Heparinized blood samples were transferred to a 40-mm glass capillary tube sealed with plasticine or soap at both ends and centrifuged at 2520 *g* for 5 min. Hematocrit (Hct) was calculated using the ratio of erythrocyte length and total length in the glass capillary tube. Heparinized blood samples were transferred to an 80-mm glass capillary sealed with plasticine or soap at the bottom. Erythrocyte sedimentation rate (ESR) was determined in a sedimentation unit. Measurements were performed at room temperature.

The red blood cells (RBC) used for ESR detection were added to the electrophoresis plate, and the electrophoresis or electrode buffer was added to the electrophoresis tank with suction tubes or to the electrode tank with a sample injector, respectively. The maximum and minimum ranges of RBC were measured to calculate erythrocyte electrophoretic mobility. Erythrocyte deformability was calculated using the analysis system parameters of the 3-9D type microcirculation, hemorheology and erythrocyte deformation integrated analyzer according to the indices of whole blood viscosity, plasma viscosity and Hct.

Statistical analysis

Data are reported as means ± SD. The statistical analyses were performed using the SPSS 11.5 software. One-way ANOVA was used for comparison between groups and the paired *t*-test for within-group comparison. The level of significance was set at $P < 0.05$.

Results

Comparison of survival time

The survival time of the DIC+lymph group (263 ± 142 min) was significantly longer than that of the DIC+saline group (90 ± 53 min; $P < 0.05$).

Changes of MAP in DIC+saline and DIC+lymph groups

At the beginning of the experiment, the MAP of the DIC+saline and DIC+lymph groups was normal and did not differ significantly between groups. After Dextran 500 injection, the MAP of the DIC+lymph group increased slightly and remained steady at a higher level for 40 min, whereas the MAP of the DIC+saline group decreased slowly after 15 min, and was lower than that of the DIC+lymph group for 15-25 min (Figure 1).

Changes in the mesenteric microcirculation of DIC+saline and DIC+lymph groups

Mesenteric microvessels were clearly visible with well-defined edges and their blood stream pattern was

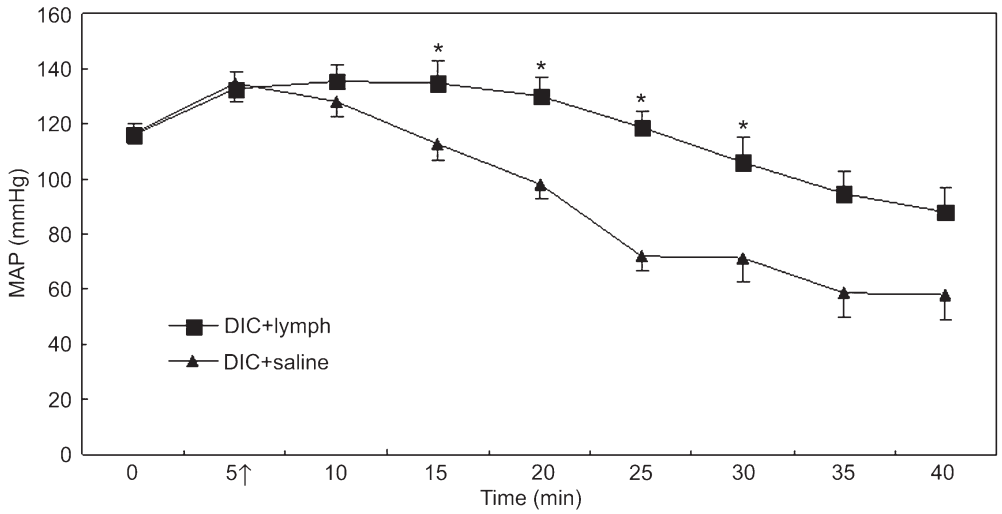

Figure 1. Effect of exogenous normal lymph on blood pressure in rats with disseminated intravascular coagulation (DIC). Lymph or saline was injected (*iv*) at 6 min (arrow). Data are reported as means ± SD for 10 rats. *$P < 0.05$ compared to the DIC+saline group (paired *t*-test).

characterized by line or line-granular flow before Dextran 500 injection in both the DIC+saline and DIC+lymph groups, and no RBC aggregation was observed (Figure 2A and G). Injection of Dextran 500 significantly blunted the rate of blood flow and the blood stream showed granular or slow granular flow (Figure 2B and H). However, administration of normal lymph significantly ameliorated the microcirculatory disturbances and its action lasted for more than 40 min (Figure 2C-F and I-L). The integral values for different microvessel levels were lower in the DIC+lymph group than in the DIC+saline group (Figure 3).

The initial caliber of mesentery blood vessels did not differ significantly between DIC+saline and DIC+lymph groups before the experiment (Table 2). The application of Dextran 500 significantly reduced the caliber of A_1, A_2, V_1, and V_2 after 5 min. However, the calibers of A_1, A_2, V_1, and V_2 increased significantly after the administration of normal lymph at the observation time points of 10, 15, and 20 min, and were wider than those of the DIC+saline group (Figure 4).

Changes in blood viscosity in the three groups of rats

Whole blood viscosity at high, medium and low shear rates increased significantly in the DIC+saline group compared to control, and decreased in the DIC+lymph group compared to the DIC+saline group, although they did not differ significantly from control (Figure 5). The relative viscosity of whole blood was higher in the DIC+saline group and lower in the DIC+lymph group compared to control, and lower in the DIC+lymph than in the DIC+saline group; the reduced viscosity of whole blood was lower in the DIC+lymph group than in the DIC+saline and control groups (Figure 6). Plasma viscosity was higher in the DIC+saline (1.72 ± 0.13 mPa s) and DIC+lymph (1.66 ± 0.17 mPa s) groups than in the control group (1.22 ± 0.12 mPa s), with no statistically significant difference between the DIC+saline and DIC+lymph groups.

Changes of Hct in the three groups of rats

The Hct of the DIC+lymph (50.68 ± 2.80%) and DIC+saline (49.57 ± 2.98%) groups was significantly higher than that of the control group (40.25 ± 3.29%), and the Hct of the DIC+lymph group was higher than that of the DIC+saline group, although the differences between them were not statistically significant.

Changes of ESR indices in the three groups of rats

After Dextran 500 injection, the ESR, K value of the equation and K value of emendation in the DIC+lymph and DIC+saline groups were significantly higher than in the control group, but did not differ significantly from one another (Figure 7).

Changes of erythrocyte electrophoresis in the three groups

After Dextran 500 injection, the electrophoresis time of erythrocytes was markedly longer in the DIC+lymph and DIC+saline groups than in the control group, but the electrophoresis length of erythrocytes and erythrocyte migration were significantly lower in the DIC+lymph and DIC+saline groups than in the control group and did not differ significantly between the DIC+lymph and DIC+saline groups (Figure 8).

Changes of erythrocyte deformability in the three groups

There were no statistically significant differences in erythrocyte deformability between the DIC+saline and control groups at different shear rates. However, erythrocyte deformability was remarkably enhanced in the DIC+lymph group compared to the other two groups (Figure 9).

Discussion

Studies have shown that losses of intestinal lymph can result in a significant decrease of blood pressure even with the addition of albumin and intralipid equivalent to the amount lost (7). However, through the bypass of intestinal lymphatic to jugular vein, reinfusion of lymph avoids the decline in blood pressure (7). Moreover, a small amount of lymph from the intestine or thoracic duct of rats, dogs or rabbits exerts a significant pressor effect on severe hemorrhagic shock, which is similar for different animal species (2,8). It has been reported that the lymph from healthy dogs has a protective role in blood pressure and can reduce the myeloperoxidase (MPO) activity of lung tissue in rats subjected to endotoxic shock (9,10). This evidence suggests that lymph has an important role in the maintenance of blood pressure. Thus, in the present study, we used normal lymph from dogs for intervention in DIC rats, and we found that a small amount of exogenous normal intestinal lymph had an obvious pressor effect on DIC rats, since the amount of intestinal lymph is only 1/15 of total blood volume, which is hard to explain by supplementation of blood volume. These results indicate that, besides acting as a supplement for blood volume, the pressor effect of lymph from healthy animals may be involved in the regulation of cardiac function and vascular resistance. The results demonstrate that normal lymph can improve the contractile function of the heart in rats insulted by severe hemorrhagic shock (11).

The mechanism of DIC induction by macromolecular Dextran 500 is, on the one hand, related to the activation of the intrinsic coagulation pathway (12,13). On the other hand, Dextran 500 can form a single-molecule layer among the erythrocytes and adhere to the surface of both ends of the macromolecules forming a bridging. Simultaneously, fibrinogen, globulin and other protein

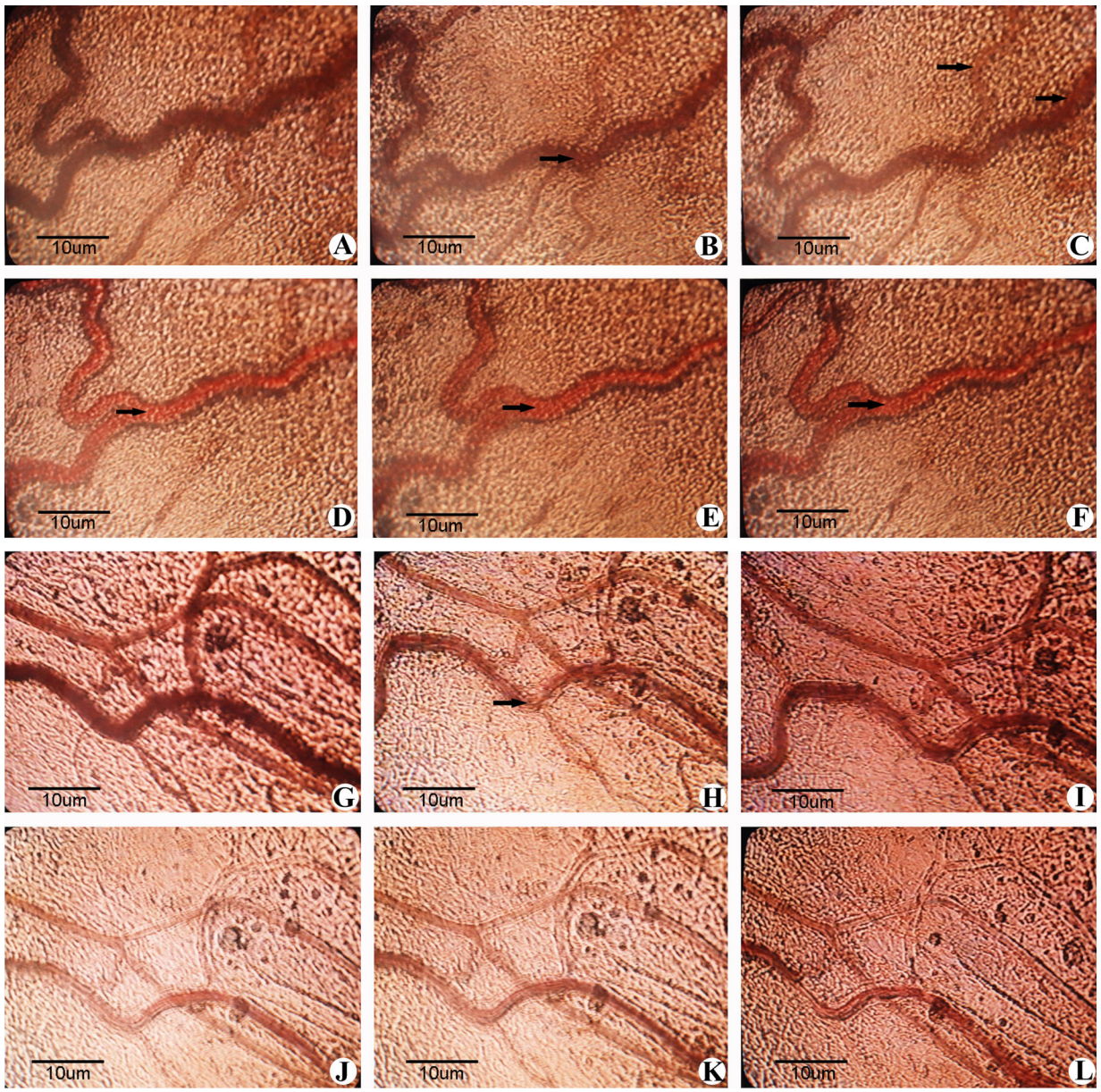

Figure 2. Vital micrograph of the microcirculation in the DIC+saline and DIC+lymph groups. *A*, Before administration of Dextran 500 to the DIC+saline group; *B*, 5 min after administration of Dextran 500 to the DIC+saline group, the blood stream showed granular or slow granular flow (arrow); *C*, 10 min after administration of Dextran 500 to the DIC+saline group, the blood stream showed granular or slow granular flow (arrows); *D*, 20 min after administration of Dextran 500 to the DIC+saline group, the blood stream showed vibratory granular flow and moderate erythrocyte aggregation (arrow); *E*, 30 min after administration of Dextran 500 to the DIC+saline group, the blood stream showed vibratory granular flow and moderate erythrocyte aggregation (arrow); *F*, 40 min after administration of Dextran 500 to the DIC+saline group, the blood stream showed vibratory granular flow and moderate erythrocyte aggregation (arrow); *G*, before administration of Dextran 500 to the DIC+lymph group; *H*, 5 min after administration of Dextran 500 to the DIC+lymph group, the blood stream showed granular or slow granular flow (arrow); *I*, 10 min after administration of Dextran 500 to the DIC+lymph group; *J*, 20 min after administration of Dextran 500 to the DIC+lymph group; *K*, 30 min after administration of Dextran 500 to the DIC+lymph group; *L*, 40 min after administration of Dextran 500 to the DIC+lymph group.

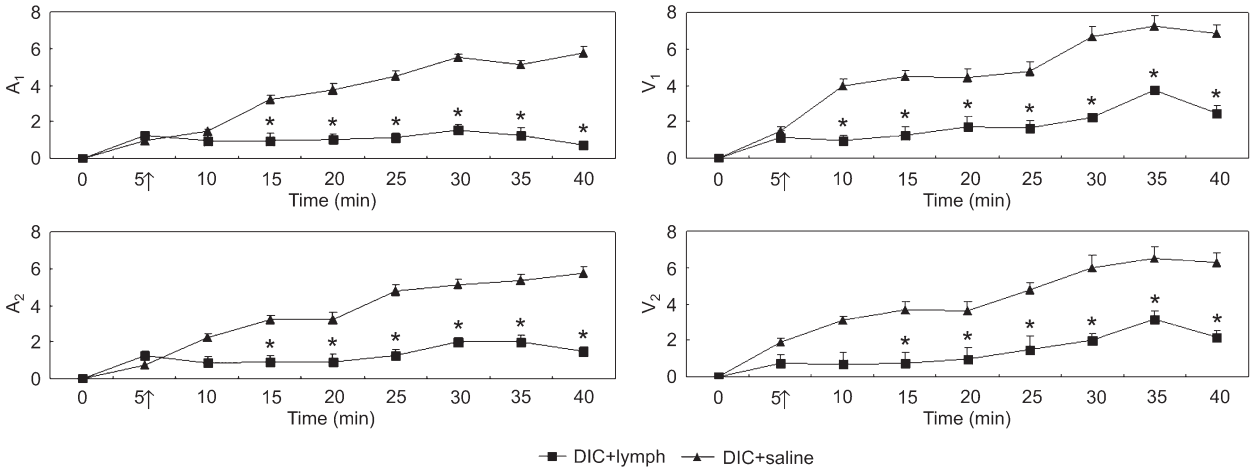

Figure 3. Effect of exogenous normal lymph on blood flow conditions in rats with disseminated intravascular coagulation (DIC). Lymph or saline was injected (*iv*) at 6 min (arrow). Data are reported as means ± SD for 10 rats. A_1 = first-order arterioles; A_2 = second-order arterioles; V_1 = first-order venules; V_2 = second-order venules. *P < 0.05 compared to the DIC+saline group (paired *t*-test).

Table 2. Basal values of arteriole and venule diameters.

Group	A_1	A_2	V_1	V_2
DIC+lymph group	20.30 ± 1.79	12.69 ± 3.87	26.69 ± 10.13	18.43 ± 8.13
DIC+saline group	18.20 ± 2.70	14.56 ± 2.70	25.11 ± 6.59	13.24 ± 3.89

Data are reported as means ± SD in μm for 10 rats. A_1 = first-order arterioles; A_2 = second-order arterioles; V_1 = first-order venules; V_2 = second-order venules. There were no significant differences in these indices between the DIC+lymph and the DIC+saline groups.

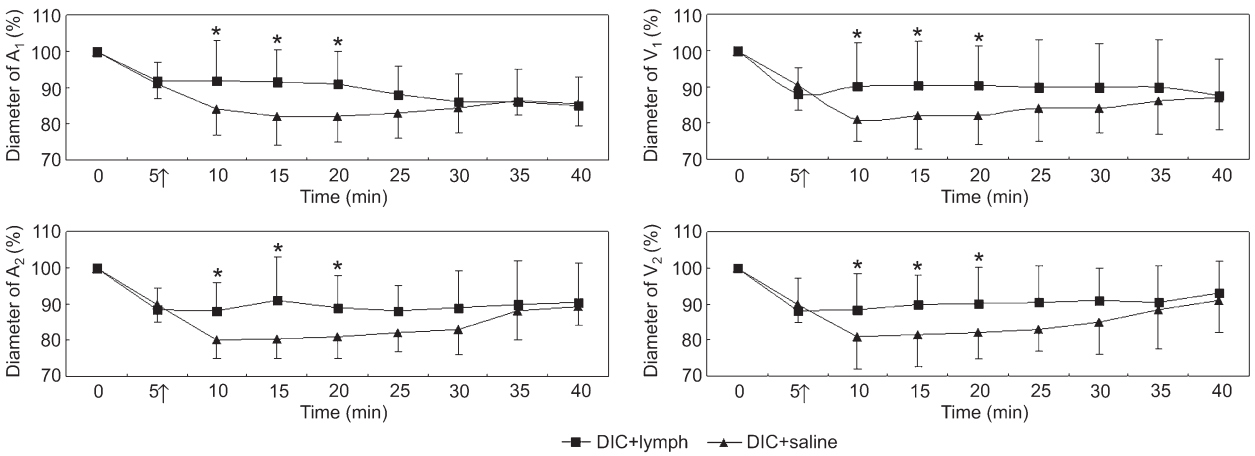

Figure 4. Effect of exogenous normal lymph on microvessel diameter in rats with disseminated intravascular coagulation (DIC). Lymph or saline was injected (*iv*) at 6 min (arrow). Data are reported as means ± SD for 10 rats. A_1 = first-order arterioles; A_2 = second-order arterioles; V_1 = first order venules; V_2 = second order venules. *P < 0.05 compared to the DIC+saline group (paired *t*-test).

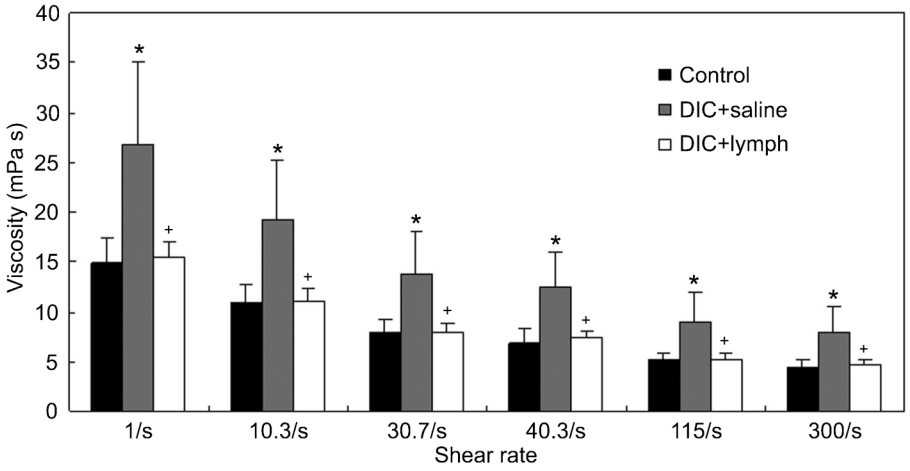

Figure 5. Changes of whole blood viscosity in the three rat groups studied. Data are reported as means ± SD in mPa s for 10 rats. *P < 0.05 compared to the control group; $^{+}$P < 0.05 compared to the DIC+saline group (one-way ANOVA).

molecules are attached to the erythrocyte membrane. In a previous study (14), we found that the prothrombin time (PT) and activated partial thromboplastin time (APTT) were prolonged, and micro-thrombosis appeared in renal tissue. The fibrinogen (Fib) level, platelet aggregation rate (PAR), and thrombosis-forming rate *in vitro* were subsequently decreased because of consumptive coagulation.

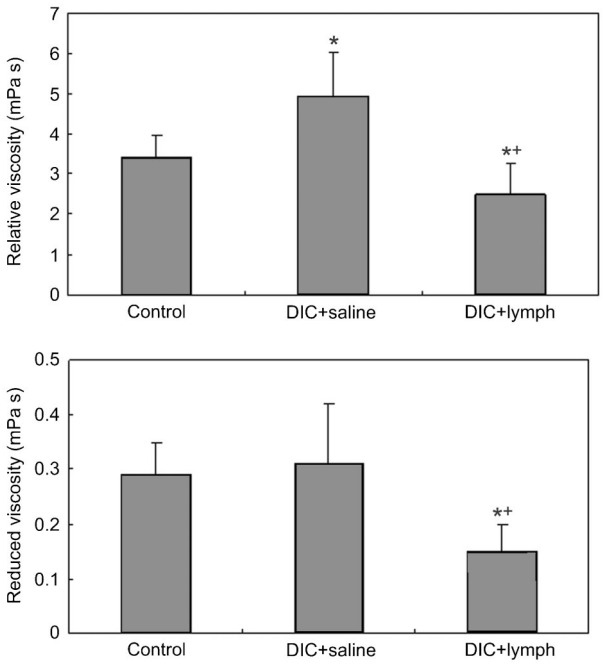

Figure 6. Changes of relative viscosity and reduced viscosity in the three rat groups studied. Data are reported as means ± SD in mPa s for 10 rats. *P < 0.05 compared to the control group; $^{+}$P < 0.05 compared to the DIC+saline group (one-way ANOVA).

These results confirm that the model of DIC was successful and consistent with clinical practice. At the same time, the changes of the PT, APTT, Fib, PAR, and thrombosis-forming rate observed in the DIC+lymph group were consistent with the DIC+saline group, although PT and APTT were more prolonged in the DIC+lymph group and PAR was more decreased than in the DIC+saline group. Therefore, the effect of lymph in improving DIC is involved in decreasing consumptive coagulation although this mechanism needs to be further confirmed.

In the present study, these changes in the erythrocytes resulted in a reduction of electric repulsive force, membrane strain and mechanical shearing, which are all disaggregation forces that lead to erythrocyte aggregation (15-17). During the process of flowing in the blood stream, the erythrocytes can be easily destroyed by extrusion and cutting, with the release of pro-coagulant substances ultimately resulting in DIC. Observation of the microcirculation showed that, 5 min after the administration of Dextran 500, mean blood flow velocity was significantly reduced compared to pre-administration in both groups, with the conditions of microcirculation changing from grain or slow granular flow to vibratory granular flow, skimming or blood sludge. After treatment with a small amount of normal lymph, the microcirculation flow condition, characterized by grain flow or slow granular flow, showed a significant improvement compared to control animals. Moreover, erythrocyte aggregation and microvascular narrowing were also significantly reduced and these effects continued for more than 40 min after lymph administration. This result suggests that normal lymph can reverse erythrocyte aggregation and relieve the microvascular spasm to a certain extent, thus accelerating blood flow and markedly improving the cellular

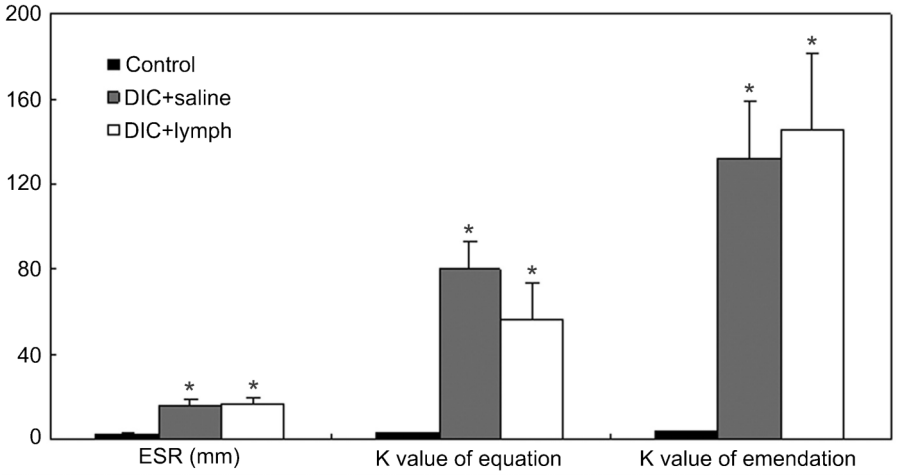

Figure 7. Changes of erythrocyte sedimentation rate (ESR) in the three rat groups studied. Data are reported as means ± SD for 10 rats. *P < 0.05 compared to the control group (one-way ANOVA).

microenvironment. These changes may contribute to the improvement of blood pressure and survival time of rats subjected to Dextran 500. Moreover, it has been documented that normal mesenteric lymph has anti-inflammatory properties that decrease the expression of intercellular adhesion molecule-1 (ICAM-1) induced by lipopolysaccharide (LPS) in the pulmonary endothelium (18). Lymph is a complex protein mixture mainly containing albumin, IgG and fibrinogen. Immunodepletion of the most abundant proteins in the mesenteric lymph demonstrated that the major functional groups in mesenteric lymph are protease inhibitors, immune-related proteins (particularly those implicated in innate immunity), carrier proteins and proteins related to the clotting system (19). These features distinguishing normal lymph from plasma may be the main contributors to its therapeutic effects.

In the present study, the hemorheological abnormalities appeared after the administration of Dextran 500 and chiefly manifested as an increase of whole blood viscosity, plasma viscosity, relative viscosity and reduced viscosity, a rise in hematocrit, an accelerated erythrocyte sedimentation rate, and an increased K value. The main reasons for these changes were: 1) an insufficient oxygen supply due to microcirculatory disturbance can increase local capillary permeability, result in blood concentration and lead to the enhancement of blood viscosity and

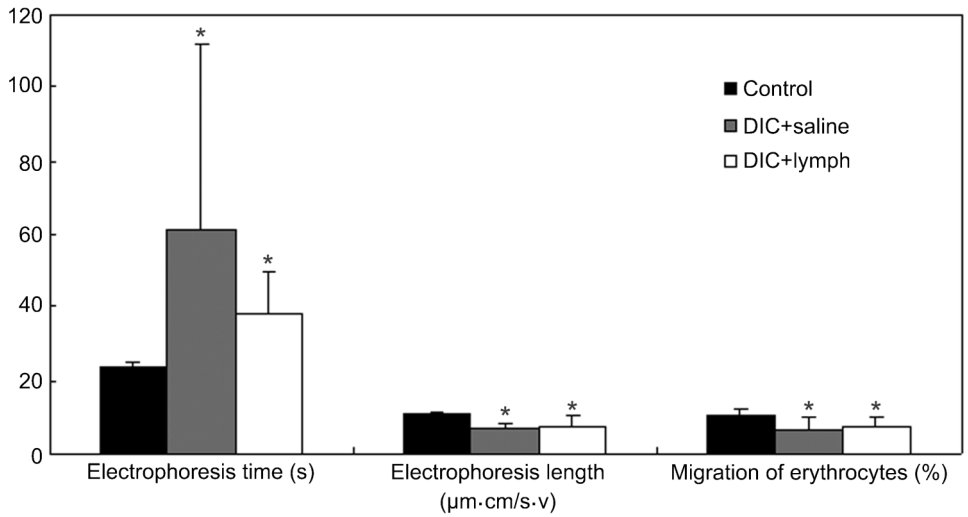

Figure 8. Changes of the electrophoretic properties of erythrocytes in the three rat groups studied. Data are reported as means ± SD for 10 rats. *P < 0.05 compared to the control group (one-way ANOVA).

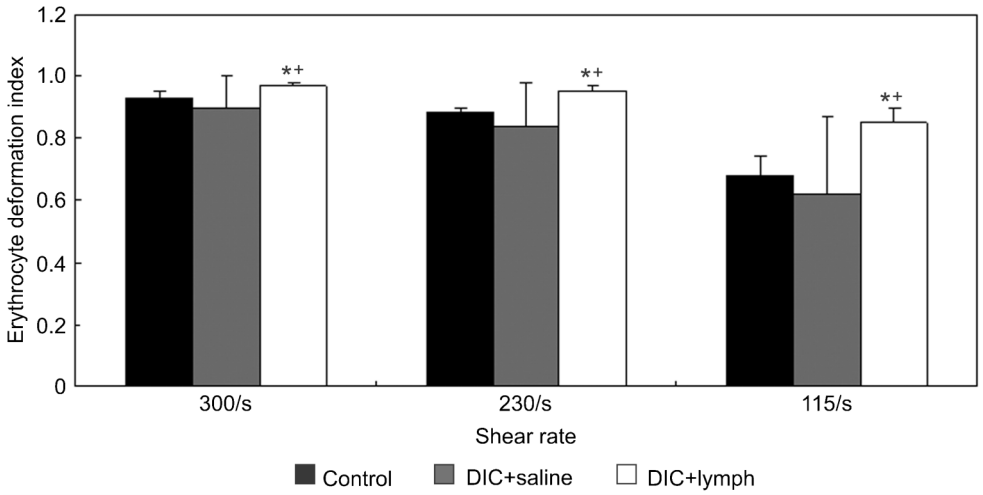

Figure 9. Changes of erythrocyte deformability in the three rat groups studied. Data are reported as means ± SD for 10 rats. *$P < 0.05$ compared to the control group; $^{+}P < 0.05$ compared to the DIC+saline group (one-way ANOVA).

ultimately flow resistance. 2) The decline of cardiac output induced by decreased returned blood volume can aggravate the hemorheological abnormalities. After the administration of normal lymph, the whole blood viscosity was significantly reduced and the plasma viscosity showed no obvious improvement. The mechanism of action of normal lymph on whole blood viscosity is related to improvement of RBC deformability, which was confirmed in the subsequent experiments. Because Fib levels can affect plasma viscosity but injection of normal lymph could not influence the Fib level, there was, therefore, no statistical difference between DIC+lymph and DIC+saline groups. Thus, it is clear that these effects may be mainly related to the changes in cellular function. Erythrocyte deformability is a key factor influencing blood viscosity. Schwarz-Benmeir et al. (20) reported that the erythrocytes of old individuals had lower amounts of calpastatin and less calpastatin activity than those of young individuals and thus calpain binding and activation were enhanced in the erythrocyte membranes, along with enhanced degradation of band 3 (a major erythrocyte transmembrane anion-transport protein) and reduced activity of Ca^{2+}-ATPase (21). Enhanced activation of erythrocyte calpain can directly dissolve the cytoskeletal proteins of the membrane and reduce erythrocyte deformability (22). Decreased Ca^{2+}-ATPase activity can result in the elevation of intracellular and membrane Ca^{2+} concentration, with a consequent increase of membrane stress and decrease of deformability. Simultaneously, enhanced activation of erythrocyte calpain and degradation of calpastatin occur under conditions of increased cellular Ca^{2+} (20) and aggravate the injury to Ca^{2+}-ATPase and membrane skeleton proteins. The declined erythrocyte deformability is further reduced and exacerbates the hyperviscosity of blood. Erythrocyte electrophoretic time, which is negatively related to the surface charges in the erythrocyte membrane, increased significantly in the group treated with normal lymph compared to control. Erythrocyte deformability is one of the important indices that determine the rheological behavior of blood, which influences the peripheral microcirculation since RBC must deform through vessels that are smaller than their resting diameter. The decreased RBC deformability can directly retard blood flow rate, and result in stasis and occlusion. The erythrocyte deformation index was significantly enhanced compared to control in a series of shear rates from 115 to 300/s, which suggests that normal lymph can improve erythrocyte deformability. Our previous study (10) showed that a small amount of normal lymph can enhance the activity of Na^{+}-K^{+}-ATPase and Ca^{2+}-ATPase, and reduce the neutrophil sequestration of the lung and blunt the increase of blood TNF-α level in rats with endotoxic shock. These results also imply that the mechanism of erythrocyte protection by normal lymph may be related to the enhancement of membrane ATPase activity and the suppression of inflammatory responses.

In the present study, the normal lymph was derived from a healthy dog; hence, the immunological rejection between heterogeneous animals should be considered. In earlier studies from our laboratory, we found that normal lymph without leukomonocytes from a healthy dog had a positive effect on hemorrhagic shock (7,8), endotoxic shock (9,10), and DIC (14), with no immune rejection. In order to observe the possible harmful effect of foreign proteins on plasma, the plasma from a healthy dog was used as a control in early experiments. The results showed that there were no microcirculation disorders or immunological rejection to the foreign protein from dog

plasma, and in the present study, the rejection response was also not found. These results confirmed that dogs are safe as donors. However, the long-term effect of normal lymph from heterogeneous animals needs further observation.

In summary, exogenous normal lymph can affect the acute microcirculation disturbance and abnormal hemorheological properties, and maintain blood pressure in rats with DIC induced by Dextran 500. These findings could provide a new treatment for patients with DIC induced by septic shock or other factors. It should be noted that the lymph volume was only 1/15 of the whole blood volume, so the effects are difficult to explain by supplement of blood volume and hemodilution. These data also indicate that normal lymph decreases the negative effects induced by volume resuscitation and could contribute to the development of new solutions for volume replacement. However, it is not clear which substances in normal lymph result in such beneficial protection. With the application of proteomic and metabonomic technologies, it is believed that answers can be found and, of course, this is the direction of our further investigations.

Acknowledgments

Research supported by the National Natural Science Foundation of China (#30370561, #30770845, #30971203) and the Natural Science Foundation of Hebei Province (#C2004000649, #C2008000503).

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