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Heterogeneity of capillary flow in the retrograde microcirculation induced in rat limb by arteriovenous shunting

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Abstract

Arteriovenous (AV) fistulas have been used clinically for improving adjunctive bypass patency. Such AV shunting induces retrograde flow in the microvascular network, which may induce microvascular remodeling and angiogenesis at the chronic phase. This paper was aimed to examine heterogeneity of blood flow among capillaries in the retrograde microcirculation induced by AV shunting. An AV anastomosis was created in rat hind limb. Using a dual window method or frame-by-frame technique on the fluorescence microscopic video images, we measured velocities of red blood cells (RBCs) flowing in the capillary network in three flow conditions: control (normal flow), arterial occlusion, and AV shunting (retrograde flow). For each flow condition, RBC velocities were obtained in 155 capillaries of 6 rats. By classifying all the capillaries into four groups based on the levels of RBC velocity in the occlusion state, we evaluated the mean velocities, coefficient of variation (CV), and histograms for each group of capillaries. The mean velocity and CV in each group changed significantly from the control to AV shunting states. Especially, most significant changes appeared in capillary groups where the superficial femoral artery or its collateral arteries might have a direct influence. Though the AV shunting improved capillary perfusion in the mean level, major parts of capillaries still remained at low perfusion.

Keywords: Arteriovenous shunting; Capillary network; Fluorescence video microscopy; Heterogeneity; Limb microcirculation; Red blood cell velocity; Retrograde flow

Introduction

Treatments using arteriovenous (AV) fistulas have been used for improving adjunctive bypass patency (Dardik et al., 1991; Paty et al., 1990). Such AV shunting induces various changes in the microvasculature. At the acute phase, hemodynamic changes such as retrograde flow or heterogeneity of red blood cell (RBC) perfusion appear in the capillary network (Komai et al., 2000; McGovern et al., 1985; Nakano et al., in press). At the chronic phase, adaptations such as vascular remodeling and angiogenesis appear in responses to hemodynamic or metabolic change (Hershley et al., 2001; Herzog et al., 2002; Komai et al., 2005; Murant and Sarelius, 2000).

In the retrograde microcirculation induced by AV shunting, collateral vessels may develop as draining vessels, so that arterial blood flows within the capillary network from the vein, draining into the collateral vessels. Such flow circuits are non-physiological or artificial. The microvascular hemodynamics may show different features from those in the normal (normograde) microcirculation. The flow distribution in the capillary network may not be predicted based on those in the normograde microcirculation.

It is well known that flow distribution is never homogeneous within the capillary network but heterogeneous among capillaries. The heterogeneity (spatial and temporal) is influenced by a number of factors. Among them, the major factors are capillary network topology (Ellis et al., 1994; Groom et al., 1995; Pries et al., 1995, 1996) and flow-rate (Tyml and Mikulash, 1988; Tyml et al., 1995). These factors have not yet been verified in non-physiological retrograde microcirculation.

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Up to now, a number of studies have indicated that wall shear stress is responsible for microvascular adaptation at the chronic phase (Hudlicka, 1998; Milkiewicz et al., 2001). The wall shear stress is closely related with the flow distribution in the capillary network. However, there are few data available for heterogeneity with low or high perfusion capillaries in the retrograde microcirculation.

In our previous paper (Nakano et al., in press), we studied capillary flows in the retrograde microcirculation induced in rat limb by AV shunting. By measuring velocities of RBCs flowing in the capillary network, we found that AV shunting increased capillary perfusion with increasing low and high perfusion capillaries. In this paper, we re-examined the spatial heterogeneity in the AV shunt-induced retrograde microcirculation in views of classified capillaries. By evaluating the mean velocity, coefficients of variation, and histogram of RBC velocities for each capillary group, we examined the local heterogeneity in the retrograde microcirculation.

Materials and methods

Animal preparation with AV anastomosis

Six male Wistar rats (240–280 g b.w.) were used in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (published by the Physiological Society of Japan). The animals were anesthetized with thiobutabarbital sodium (100 mg/kg i.p.) and tracheotomized for mechanical ventilation. The jugular vein, carotid artery, and left femoral artery were cannulated for drug infusion, continuous recording of pressure, and sampling/transfusion of blood, respectively. The gracilis and sartorius muscles of the right pelvic limb were exposed with groin and lateral incisions. The muscle membranes were removed to get a clear view of the superficial microvascular network. The muscle surface was superfused with phosphate buffer solution (PBS; pH 7.40) throughout the experiments. Blood was sampled before and after each recording, and the pH, pO_2, and pCO_2 of blood were adjusted to 7.37–7.42, 95–105, and 35–40 mm Hg, respectively. The blood pressure was maintained around 120 mm Hg by transfusing the same volume of the sampled blood. The animal was allowed to stabilize for 30 min before each recording.

AV anastomosis was performed according to the surgical procedure reported in previous papers (Komai et al., 2005, in press). Briefly, the right superficial femoral artery (SFA) and vein (SFV) were exposed with a 3-cm incision on the skin along the vessels. Both the vessels were isolated from the surrounding tissue between the bifurcation of profunda femoral arteries (PFAs) or veins (PFVs) and their next bifurcation. Both vessels were ligated at the downstream sites and clipped at the upstream sites. Both vessels were dissected completely near the ligations. By making a transverse incision on the artery and vein, a polyethylene tube (3 mm in length) was inserted into both vessels. After the inserted tube was secured with sutures, the incised vessels were disconnected completely (see Fig. 1).

Reperfusion was performed with 3–4 ml blood withdrawal/injection through the femoral artery (FA). The time duration of operation was about 30 min, and the time duration of ischemia was 20 min.

Intravital fluorescence video microscopy and red blood cell velocity measurement

Red blood cells (RBCs) labeled with fluorescein 5-isothiocyanate (FITC; Sigma, USA) were injected intravenously to visualize the limb microcirculation; the injection volume was selected such that one cell passed per 1 s in

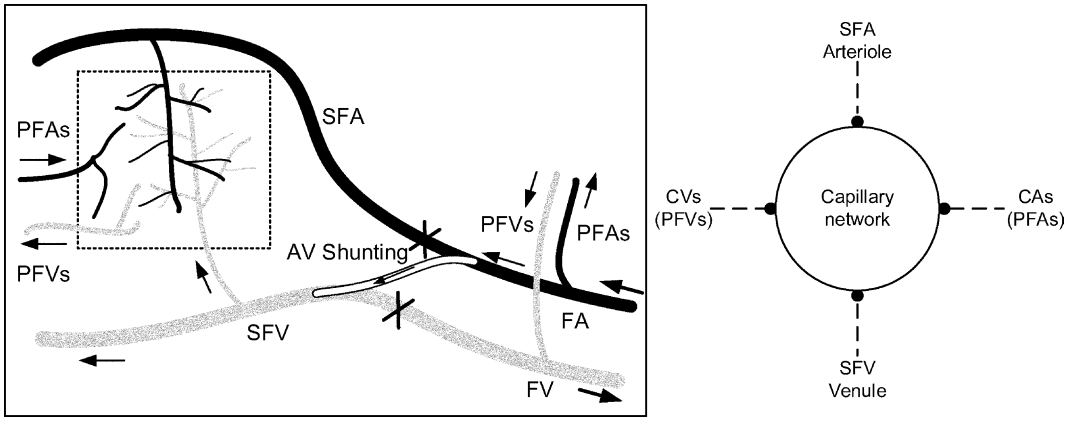

Fig. 1. Illustrations of blood circulation in the vascular network of rat hind limb in AV shunting condition (SFA, superficial femoral artery; SFV, superficial femoral vein; PFAs, profunda femoral arteries; PFVs, profunda femoral veins; FA, femoral artery; FV, femoral vein; arrows, direction of flow) (left) and a capillary network model (CAs, collateral arterioles; CVs, collateral veins) (right). In the vascular network (left), the rectangular window indicates the site for microscopic observation and measurement. In the capillary network model (right), the CAs and CVs are connected with PFAs and PFVs, respectively. In the control (normal) state, blood flows through the capillary network from arterioles (connected with SFA) to draining venules (connected with SFV) while the CAs and CVs are closed. In the AV shunting condition, blood flows through the capillary network from the SFV or CAs to the CVs (see text).