Evaluation of the Dynamics of Choroidal Circulation in Experimental Acute Hypertension Using Indocyanine Green-stained Leukocytes

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Purpose: To evaluate the dynamics of choroidal circulation in experimental acute hypertension, using the indocyanine green leukocyte angiography (ILA) method, which the authors have developed for the evaluation of leukocyte dynamics in choroidal circulation.

Methods: Japan White rabbits were used in the present study. Leukocytes were collected by centrifugal separation of the autologous blood, and were stained with indocyanine green (ICG) dye. The ICG-stained leukocyte fluid was injected into an ear vein, and fundus images were obtained by infrared laser and a scanning laser ophthalmoscope. Experimental acute hypertension was induced by the intravenous drip injection of angiotensin II (AII).

Results: The fluorescent dots rapidly moved in choroidal arteries at a decreasing velocity, passed very slowly through choroidal capillaries and drained into choroidal veins. Under normal blood pressure, the mean leukocyte velocities in arteries, capillaries and veins were 8.63 ± 1.68, 0.52 ± 0.07, and 6.96 ± 2.20 mm/s, respectively. On the other hand, the respective mean velocities in acute hypertension induced by AII were 13.50 ± 1.82, 0.81 ± 0.09, and 10.54 ± 3.91 mm/s. Besides flow velocity, no change in leukocyte dynamics was observed.

Conclusions: Under the condition of acute hypertension induced by AII, leukocytes moved faster in the total choroidal circulation (from arteries to veins) compared to their velocity under the condition of normal blood pressure. Blood velocities might increase in the total choroidal circulation at an early stage in acute hypertension induced by AII, resulting in increased choroidal blood flow. ILA makes it possible to evaluate the changes in choroidal circulation under various pathologic conditions. **Jpn J Ophthalmol 2003;47:572–577** © 2003 Japanese Ophthalmological Society

Key Words: Angiotensin II, choroidal circulation, hypertension, indocyanine green angiography, leukocyte.

Introduction

The choroidal circulation under physiological and pathological conditions has been studied in vivo by the use of various methods, such as the hydrogen clearance method, laser Doppler flowmetry, and laser speckle phenomenon.[1–7] Although it is known that hypertension causes various changes in the circulation system of the whole body, there have been few studies on choroidal circulation in experimental systemic hypertension. Fujimoto reported choroidal blood flow unchanged in acute hypertension induced by angiotensin II (AII) by means of the hydrogen clearance method.[8] Yamaguchi and associates investigated the effects of kallidinogenase on choroidal blood flow in a renal hypertensive rabbit model.[9]

Leukocytes, one of the circulating components, are known to play a key role in the microcirculation under certain pathological conditions.[10–13] There have been in vitro studies on the activation of peripheral blood monocytes in acute hypertension induced by AII, which may

lead to increased monocyte binding to vascular endothelial cells and subendothelial infiltration of monocytes.[14,15] To our knowledge, however, there have not been any reports on leukocyte dynamics in experimental hypertension. Recently, we developed a new method, indocyanine green leukocyte angiography (ILA), which makes possible direct visualization of leukocytes moving in the choroidal vessels of albino rabbits, pigmented rabbits, and monkeys, and enables a researcher to analyze leukocyte dynamics in the choroidal circulation through autologous leukocytes stained with indocyanine green (ICG) dye.[16] Thus, we evaluated the change in choroidal circulation dynamics in rabbits using ILA after experimental acute hypertension was induced by AII. ILA might become available for clinical use because the low concentration of ICG used in this method is not toxic.

Materials and Methods

Animal Preparation

Twenty rabbits (Japan White), weighing 2.8–4.0 kg, were used. All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Pentobarbital sodium (25 mg/kg; Nembutal, Abbott Laboratories, North Chicago, IL, USA) was used for anesthesia by injection through an ear vein and was supplemented as needed during the experiment.

Indocyanine Green Leukocyte Angiography

Fifteen milliliters of blood was withdrawn from an ear vein into a sterile test tube. The blood was mixed with a mixture of Ficoll and metrizoate (Mono-Poly Resolving Medium, Dainippon Pharmaceutical, Osaka), and separated using a centrifuge at 1800 rpm for 30 minutes. Most of the plasma and erythrocyte bulk was removed. The separated white-coat layer of leukocytes was mixed with 0.25 mg/mL ICG solution (Diagnogreen injection, Daiichi Pharmaceutical, Tokyo), and centrifugal separation of the leukocyte fluid at 1500 rpm for 5 minutes was additionally performed to collect a denser concentration of ICG-stained leukocytes. Phosphate-buffered saline was added to the ICG-stained leukocyte fluid. About 6 mL of leukocyte fluid was estimated to contain 30–90 million leukocytes by counting the leukocytes in 0.1 μL of the leukocyte fluid.

Pupils were dilated with 0.5% tropicamide. The ICG-stained leukocyte fluid was injected into an ear vein under normal tension or constant high pressure during the administration of AII. The fundus images were obtained with infrared laser and a scanning laser ophthalmoscope (SLO model 101; Rodenstock Instrument, Munich, Germany). The images obtained were recorded on S-VHS videotapes.

Induction of Experimental Acute Hypertension

We prepared 5 μmol/L AII solution (Angiotensin II Human; Peptide Institute, Osaka). Twenty minutes after the injection of the leukocyte fluid, 10 rabbits were given a drip injection of the AII solution at the rate of 1 mL/min. The other 10 rabbits were given physiological saline at the same rate, and served as the control.

Monitoring of Blood Pressure

A 24-gauge short catheter (Angiocath; Becton Dickinson Vascular Access, Sandy, UT, USA) was inserted into an ear artery. The catheter was connected to a pressure amplifier (AP-620G; Nihon Kohden, Tokyo) through a pressure transducer (DX-312; Nihon Kohden). The blood pressure was measured and recorded continuously with a pen recorder (VP6523A; Panasonic, Tokyo). The heart rate was simultaneously recorded.

Data Analysis

The recorded images were analyzed with an image analysis system. Images were captured with an analogue-digital converter board (Dig98; Ditect, Tokyo) and loaded into a computer (PC9821Xa; NEC, Tokyo). Images of consecutive frames were superimposed with the use of original software running on Windows 95 (Microsoft Japan, Tokyo) to trace the movement of fluorescent leukocytes in one picture. The center position of fluorescent dots was marked on a computer monitor and the distance of consecutively marked positions was measured in pixels as a spline curve.[17] The real size was determined by inserting a thin ruler along the surface of the retina using the SLO to obtain measurements, and the leukocyte velocity, which was calculated in pixels from the distance of consecutive positions, was converted into the actual value. Under normal pressure before the administration of AII and under constant high pressure during the administration of AII, the velocities of 15 leukocytes were measured in choroidal circulation from the artery through the capillaries to the vein in each animal. The diameters of the arteries and the veins were measured and compared by using a digital technique reported by Tsuchida and associates.[18] The measurements were performed in the midperipheral region inferior to the optic disc.

Results

After injection of the leukocyte fluid, a transit phase similar to regular ICG angiography was seen for 3 minutes. Choroidal arteries and veins were outlined as negative features against the background and fluorescent dots

were seen to be moving in the choroidal circulation. Although the movement of fluorescent dots did not always show a similar pattern, most of these fluorescent dots rapidly passed through the arteries and moved into the capillaries at a decreasing velocity. The dots slowly passed through the capillaries, and then moved into the veins at an increasing velocity (Figure 1a). Although arteries and veins gradually became obscure, we could determine where these dots were moving, in the artery, capillary, or vein, based on the changes in velocity and movement (Figures 1b and 2). The blood pressure before the administration of AII was 88.1 ± 8.1 mm Hg (mean $\pm$ SD), and increased to 155.3 ± 20.4 mm Hg during the administration of AII. In the control group, the blood pressure was constant before and during the administration of physiological saline (91.8 ± 9.9 mm Hg). The heart rate ranged from 180 to 280/min under physiological conditions in both groups. In contrast, the mean heart rate decreased from 230 to 100/min during the administration of AII.

Changes in Leukocyte Dynamics Under Acute Hypertension

The fluorescent leukocytes moved faster throughout the choroid under acute hypertension than under normal blood pressure. Although we paid attention to trapping, rolling of leukocytes in choroidal veins and firm adhesion to vascular walls, there was no apparent change in leukocyte dynamics besides the flow velocity after the administration of AII.
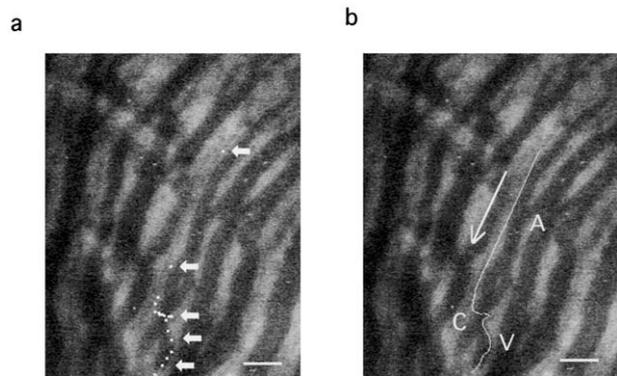
Figure 2. Typical velocity change for leukocytes in the choroidal circulation.

In Choroidal Capillaries

The mean flow velocity of leukocytes was 0.52 ± 0.07 mm/s under normal blood pressure and 0.81 ± 0.09 mm/sin acute hypertension during the administration of AII (Figure 3). There was a statistically significant difference ($P < .001$, paired t-test). In the control group, the mean flow velocity was 0.53 ± 0.07 mm/s under normal blood pressure and 0.52 ± 0.07mm/s during the administration of physiological saline.

Figure 1. Representative images obtained by using an indocyanine green leukocyte angiography method developed by the authors to evaluate leukocyte dynamics in choroidal circulation. (**a**) Composite photograph of consecutive images demonstrating a fluorescent dot moving in the choroidal circulation. (**b**) Passage of the fluorescent leukocyte that was consecutively traced as spline curves. Arrow shows direction of flow. A: artery; C: capillary; V: vein. Bars = 0.2 mm.
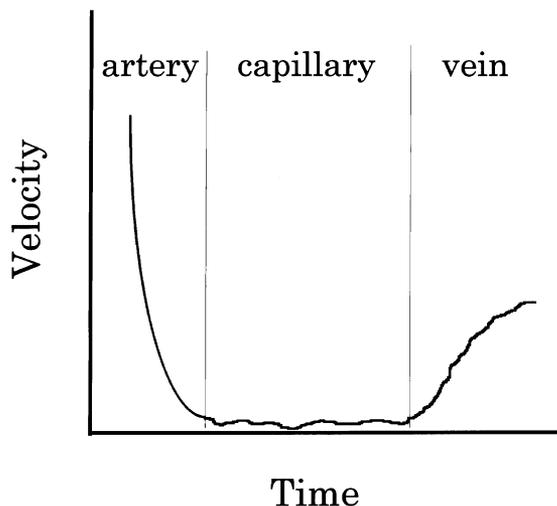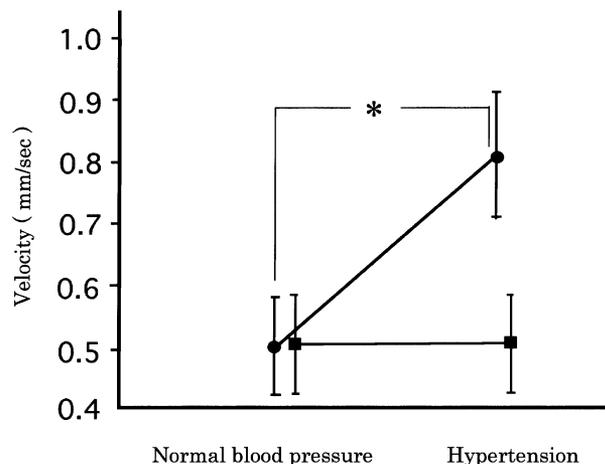
Figure 3. Changes in the mean flow velocity of leukocytes in choroidal capillaries. ●: Angiotensin II injection, ■: control. The error bars represent the standard deviation of the mean. The asterisk indicates significant difference between the two groups ($P < .001$, paired t-test).

In Choroidal Arteries

Although the fluorescent dots moved fast in arteries, the velocity could be measured on images of three consecutive frames immediately before the entry into capillaries (Figure 4). In the AII group, the mean flow velocity of leukocytes was 8.63 ± 1.68 mm/s under normal blood pressure and 13.50 ± 1.82 mm/s during AII administration. There was a statistically significant difference ($P < .001$, paired t-test). There was no change in the trapping of leukocytes at the entry to capillaries during the experiment. During AII administration, the diameter of arteries decreased 52.6% to 95.2% with a median of 67.1% of that before AII administration. In the control group, the mean velocity was 8.89 ± 1.53 mm/s under normal blood pressure and 9.12 ± 2.18 mm/s during the administration of physiological saline. There was no significant difference. There was no change in the diameter of arteries in the control group during the experiment.

In Choroidal Veins

We measured the velocity of the fluorescent dots moving in the central position of veins. The mean flow velocity of leukocytes was 6.96 ± 2.20 mm/s under normal blood pressure and 10.54 ± 3.91 mm/s during AII administration. There was a statistically significant difference ($P = .004$, paired t-test). In the control group, the mean velocity was 7.07 ± 1.73 mm/s under normal blood pressure and 7.08 ± 2.11 mm/s during the administration of physiological saline. There was no significant

difference. There was no change in the diameter of veins after the administration of AII. During the experiment, there was no detectable rolling of leukocytes in the choroidal veins (Figure 5).

Discussion

Hypertension is one of the important diseases that can lead to pathological changes in the retina and choroid. Although there have been many reports on retinal and choroidal circulation in hypertension, there are no reports describing leukocyte dynamics in the ocular circulation. AII is recognized as an important factor in the pathogenesis of hypertension. Its main function is vascular contraction through the AII receptors of arteries. In the retinal circulation, AII has a vascular contractile function, which is especially strong in small arteries.[19,20] Although these are functions of subtype 1 of AII receptors, the existence and the effect in choroidal circulation are unknown. With regard to the influence of AII on leukocyte dynamics, Kim and associates[14] demonstrated in vitro that AII might accelerate monocyte binding to vascular endothelial cells via type 1 and type 2 receptors. In the field of ophthalmology, Nadal and associates[21] showed that AII could induce migration of retinal pericytes via its type 1 receptor.

In the present study, the diameter of arteries decreased and the flow velocities of leukocytes moving in the total choroidal circulation from artery, through capillaries, to vein increased at an early stage of acute hypertension induced by AII, although we expected that the velocity might increase only in the arteries as a result of arterial
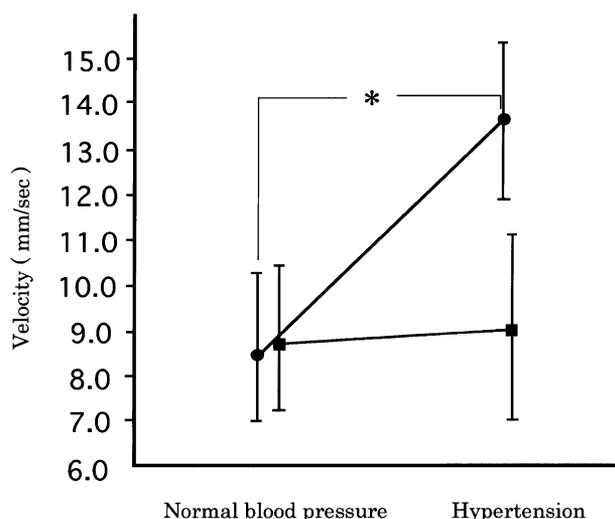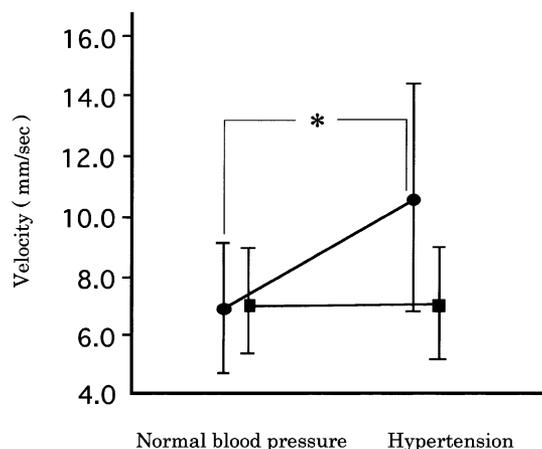

Figure 4. Changes in the mean flow velocity of leukocytes in choroidal arteries before their entry into the capillaries. ●: Angiotensin II injection, ■: control. The error bars represent the standard deviation of the mean. The asterisk indicates significant difference between the two groups ($P < .001$, paired t-test).

Figure 5. Changes in the mean flow velocity of leukocytes in the central position of choroidal veins. ●: Angiotensin II injection, ■: control. The error bars represent the standard deviation of the mean. The asterisk indicates significant difference between the two groups ($P = .004$, paired t-test).

muscular wall contraction. Our method may activate au-tologous leukocytes and change the velocity of leuko-cytes. Hossain and associates[22] reported that there was no difference in velocities between normal leukocytes and activated leukocytes, although activated leukocytes accu-mulated in the retinal capillaries and the choroidal vessels. Our results suggested that blood velocities might increase in the total choroidal circulation at an early stage in acute hypertension induced by AII, resulting in increased choroidal blood flow. The vascular resistance increases in choroidal arteries contracted by AII. The diameter of choroidal capillaries is larger than that of other organic capillaries, however, choroidal vascular resistance is less than the organic vascular resistance of other capillaries. The increase in perfusion pressure following the increase in myocardial contraction is one of the important func-tions of AII via type 1 receptors. Thus, the choroidal blood flow under the administration of AII may increase following this increase in perfusion pressure.

Because increased leukocyte–endothelium interaction is suggested as an important factor in capillary occlu-sion in various vascular diseases, leukocyte dynamics in the retinal and choroidal circulation under pathological conditions has recently been studied using acridine orange leukocyte fluorography, which can be used only in animal experiments.[23–29] In experimental diabetic models, leuko-cyte entrapment in the retinal capillaries increased with-out changing the flow velocities of leukocytes.[27,29] Thus, we paid attention to leukocyte–endothelial interaction be-sides leukocyte velocities. However, no apparent change in leukocyte–endothelial interaction, such as trapping, rolling in the vein or firm adhesion to vascular walls, was observed during the experiment. Kim and associ-ates showed, in vitro, that treatment of human endothelial cells for 18 hours with AII induced the adhesion of monocytes to these cells.[14] They reported that AII-induced monocyte binding was not associated with induc-tion of E-selectin, vascular cell adhesion molecule-1, or intercellular adhesion molecule-1. Dorfell and associ-ates[30] reported that the exposure to AII increased the secre tion of tumor necrosis factor-α or interleukin-1β in monocytes from hypertensive patients. Longer exposure of leukocytes to AII might increase leukocyte–endothelial interaction such as rolling or increased trapping.

Although further investigation is needed, this study has suggested that leukocyte velocities do increase in the total choroidal circulation in acute hypertension induced by AII. Indocyanine green leukocyte angiography will en-able us to continue our research and to evaluate leuko-cyte dynamics in choroidal circulation under pathologic conditions.

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