Leukocyte-endothelial cell interactions in diabetic retina after transient retinal ischemia

AKITAKA TSUJIKAWA,1 JUNICHI KIRYU,1 ATSUSHI NONAKA,1 KENJI YAMASHIRO,1 HIROKAZU NISHIWAKI,1 YOSHIHITO HONDA,1 AND YUICHIRO OGURA2

1Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto 606-8507; and 2Department of Ophthalmology, Nagoya City University Medical School, Nagoya 467-0001, Japan

Received 27 October 1999; accepted in final form 4 May 2000

Leukocyte-endothelial cell interactions in diabetic retina after transient retinal ischemia. Am J Physiol Regulatory Integrative Comp Physiol 279: R980–R989, 2000.—Diabetes is associated with increased neural damage after transient cerebral ischemia. Recently, leukocytes, which are thought to play a central role in ischemia-reperfusion injury, have been suggested to be involved in exacerbated damage after transient ischemia in diabetic animals. The present study was designed to clarify whether the anticipated worse outcome after transient cerebral ischemia in diabetic animals was due to augmented leukocyte-mediated neural injury. Using rats with streptozotocin-induced diabetes of 4-wk duration, we investigated leukocyte-endothelial cell interactions during reperfusion after a transient 60-min period of retinal ischemia. Unexpectedly, postischemic diabetic retina showed no active leukocyte-endothelial cell interactions during reperfusion. The maximal numbers of rolling and accumulating leukocytes in diabetic retina were reduced by 73.6 and 41.2%, respectively, compared with those in nondiabetic rats. In addition, neither preischemic insulin treatment of diabetic rats nor preischemic glucose infusion of nondiabetic rats significantly influenced leukocyte-endothelial cell interactions during reperfusion. The present study demonstrated that high blood glucose concentration before induction of ischemia did not exacerbate leukocyte involvement in the postischemic retinal injury. Furthermore, diabetic retina showed suppressed leukocyte-endothelial cell interactions after transient ischemia, perhaps due to an adaptive mechanism that developed during the period of induced diabetes.

Leukocyte-endothelial cell interactions after transient retinal ischemia (14). In addition, experimental studies have indicated increased stroke volume and worse neurological outcome. In diabetic patients, a worse outcome after transient cerebral ischemia may well be derived from augmented leukocyte-mediated neural damage during reperfusion. However, little information is available about leukocyte-endothelial cell interactions in diabetic animals after transient cerebral ischemia.

Diabetic patients have a greater incidence of cerebral ischemia than do nondiabetic subjects, and, once patients with diabetes experience a cerebral ischemic insult, they tend to have a poorer outcome. Many clinical reports support statistically the greater potential for disability and mortality of diabetic patients (11, 29, 40). However, the mechanism by which ischemic injury in diabetes is exacerbated has not been fully elucidated.

During the last decade, a growing body of evidence has indicated that leukocytes play a central role in ischemia-reperfusion injury (18). Plugged leukocytes in the postischemic microvasculature are thought to contribute to the “no-reflow” phenomenon and postischemic hypoperfusion (17). In addition, accumulated leukocytes have been reported to cause neural damage by producing proteases, superoxide radical species (33), and various kinds of inflammatory cytokines (16). The importance of leukocytes in the neural damage after transient cerebral ischemia has been shown in many experimental studies to reduce it by preventing leukocyte participation (5).

Functional and mechanical properties of leukocytes have been reported to be altered in diabetes. Leukocytes isolated from diabetic subjects are less deformable (20) and have a greater ability to generate superoxide radicals (13). Furthermore, in diabetic patients, increased numbers of circulating activated leukocytes are present in the blood and have a greater basal respiratory burst than do activated leukocytes from nondiabetic individuals (42). In addition, the expression of adhesion molecules is reportedly upregulated in venous endothelial cells of diabetic retina and choroid (22). Recently, Panés et al. (28) have reported exacerbated leukocyte behavior in the mesentery after transient ischemia. In diabetic patients, a worse outcome after transient cerebral ischemia may well be derived from augmented leukocyte-mediated neural damage during reperfusion. However, little information is available about leukocyte-endothelial cell interactions in diabetic animals after transient cerebral ischemia.
Table 1. Treatment of each group

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Preparation Before Evaluation of Leukocyte Dynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No diabetic induction</td>
</tr>
<tr>
<td>2</td>
<td>No diabetic induction, ischemia</td>
</tr>
<tr>
<td>3</td>
<td>No diabetic induction, glucose infusion, ischemia</td>
</tr>
<tr>
<td>4</td>
<td>No diabetic induction, ischemia, glucose infusion</td>
</tr>
<tr>
<td>5</td>
<td>Diabetic induction</td>
</tr>
<tr>
<td>6</td>
<td>Diabetic induction, ischemia</td>
</tr>
<tr>
<td>7</td>
<td>Diabetic induction, insulin treatment, ischemia</td>
</tr>
<tr>
<td>8</td>
<td>Diabetic induction, ischemia, insulin treatment</td>
</tr>
</tbody>
</table>

n = 6 Rats/group.

The optic media, which consist of cornea, lens, vitreous, and retina, are so transparent that the retinal microcirculation can be observed noninvasively in vivo. The retina, moreover, is part of the central nervous system, and the properties of endothelial cells as well as neural cells in the retina are reportedly similar to those in the cerebrum (8, 9, 15). We recently developed a technique of acridine orange (AO) digital fluorography that allows us to clearly visualize leukocytes in the retinal microcirculation in vivo (26, 27). Using this technique, we have shown that leukocyte-endothelial cell interactions in posts ischemic retina can be evaluated quantitatively (35–37). The study described herein was designed to evaluate leukocyte-endothelial cell interactions in diabetic retina after transient retinal ischemia to clarify whether the worse outcome after transient cerebral ischemia in diabetic patients is due to augmented leukocyte-mediated neural damage.

METHODS

Animal Model

Male pigmented Long-Evans rats (200–250 g) were used in this study. In the diabetic group, diabetes mellitus was induced by intraperitoneal injection of streptozotocin (65 mg/kg; Sigma, St. Louis, MO) diluted in citrate buffer. The nondiabetic group received the same amount of citrate buffer by intraperitoneal injection. All rats were fed standard chow ad libitum and were allowed free access to water in an air-conditioned room with a 12:12-h light-dark cycle until they were used for the experiments. All experiments were performed 4 wk after the initiation of treatment, preceded by a 12-h period without food but with free access to water.

Table 2. Physiological variables

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4 h</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC, x10⁶/µl</td>
<td>7.0 ± 0.7</td>
<td>7.1 ± 0.5</td>
<td>9.4 ± 0.7</td>
<td>10.3 ± 0.6</td>
<td>8.6 ± 0.8</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>158 ± 10</td>
<td>140 ± 8</td>
<td>137 ± 5</td>
<td>151 ± 7</td>
<td>145 ± 11</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>100.3 ± 4.0</td>
<td>101.3 ± 4.1</td>
<td>107.8 ± 5.5</td>
<td>99.8 ± 2.7</td>
<td>106.2 ± 6.2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>286 ± 4</td>
<td>282 ± 8</td>
<td>284 ± 13</td>
<td>286 ± 6</td>
<td>298 ± 14</td>
</tr>
</tbody>
</table>

Diabetic

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<table>
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<tbody>
<tr>
<td>WBC, x10⁶/µl</td>
<td>5.6 ± 0.4</td>
<td>7.8 ± 0.9</td>
<td>9.9 ± 0.4</td>
<td>10.3 ± 0.5</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>465 ± 31</td>
<td>436 ± 30</td>
<td>476 ± 15</td>
<td>435 ± 31</td>
<td>397 ± 7</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>95.8 ± 0.8</td>
<td>96.0 ± 1.8</td>
<td>98.8 ± 0.9</td>
<td>98.0 ± 3.1</td>
<td>103.7 ± 2.9</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>291 ± 11</td>
<td>279 ± 9</td>
<td>270 ± 14</td>
<td>286 ± 4</td>
<td>304 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE. WBC, peripheral leukocyte count; MABP, mean arterial blood pressure. Blood glucose level in diabetic rats was significantly higher than that in nondiabetic rats at each time point. There were no significant differences between nondiabetic and diabetic rats in other physiological parameters. *P < 0.01, †P < 0.05 compared with control values in each group. ‡P < 0.01 compared with values of nondiabetic rats at each time point.
stereotaxic platform. Body temperature was maintained at 38 ± 0.5°C throughout the experiment. Arterial blood pressure was monitored with a blood pressure analyzer (IITC, Woodland Hill, CA). AO (0.1% solution in saline) was injected continuously through the catheter for 1 min at a rate of 1 ml/min. The fundus was observed with the scanning laser ophthalmoscope in the 40° field for 5 min. At 30 min after the injection of AO, the fundus was again observed to evaluate leukocytes accumulated in the retinal microcirculation.

Experimental Design 2

Using diabetic and nondiabetic rats that had been pretreated 4 wk previously, we performed additional treatments before ischemia induction or during reperfusion (Table 1). Six rats comprised each group. In groups 1, 2, 3, and 4, nondiabetic rats were used; in groups 5, 6, 7, and 8, diabetes mellitus had been induced 4 wk before. In groups 1 and 5, leukocyte-endothelial cell interactions in nonoperated control rats were evaluated with AO digital fluorography. In all other groups, after each rat was subjected to retinal ischemia for 60 min, leukocyte-endothelial cell interactions were evaluated 12 h after reperfusion. In group 3, to evaluate the influence of acute hyperglycemia before ischemic induction on leukocyte-endothelial cell interactions during reperfusion, nondiabetic rats were treated with an intravenous injection of 3 g/kg of glucose in 0.9% saline for 1 min at 30 min before ischemic induction (41). In group 4, nondiabetic rats were treated with an intravenous injection of 3 g/kg of glucose in 0.9% saline for 1 min at 30 min before AO digital fluorography to evaluate the influence of acute hyperglycemia on leukocyte-endothelial cell interactions during reperfusion period. In group 7, diabetic rats were treated with subcutaneous injections of 0.5 U/kg of regular insulin (Eli Lilly, Indianapolis, IN) at 90 min and 0.25 U/kg at 35 min before ischemic induction to normalize the blood glucose level during the ischemic period (39). In group 8, diabetic rats were injected subcutaneously with 0.5 U/kg of regular insulin at 90 min and with 0.25 U/kg at 35 min before AO digital fluorography to evaluate the influence of acute normoglycemia on leukocyte-endothelial cell interactions during reperfusion.

In each group, leukocyte-endothelial interactions were evaluated at 12 h after reperfusion in the same way as described in Experimental Design 1.

Fig. 1. Monochromatic images of major retinal vessels in a control eye (A) and postischemic eyes at 4 (B), 24 (C), and 48 h (D) after reperfusion in nondiabetic rats. Both arteries and veins showed significant vasoconstriction immediately after reperfusion, which peaked at 4 h after reperfusion. Subsequent vasodilation occurred and peaked at 24 h after reperfusion and subsided by 48 h after reperfusion. The time course of major retinal arterial (E) and venous (F) diameters after reperfusion in each group is shown. Bar = 100 μm. Values are means ± SE. DM, diabetes mellitus. *P < 0.01, †P < 0.05 compared with control values in each group.
Image Analysis

The video recordings were analyzed with an image-analysis system, which has been described in detail elsewhere (26, 27). In brief, the system consists of a computer equipped with a video digitizer (Radius, San Jose, CA). The latter digitizes the video image in real time (30 frames/s) to 640 horizontal and 480 vertical pixels with an intensity resolution of 256 steps. To investigate inflammatory responses in the retina during reperfusion, we evaluated the diameters of the major retinal vessels, the number of rolling leukocytes in the major retinal veins, the velocity of rolling leukocytes, and the number of leukocytes accumulated in the retinal microcirculation (35–37).

The diameters of major retinal vessels were measured at 1 disc diameter from the center of the optic disc in monochromatic images recorded before AO injection. Each vessel diameter was calculated in pixels as the distance between the half-height points determined separately on each side of the density profile of the vessel image (3). The averages of the individual arterial and venous diameters were used as the arterial and venous diameters for each rat.

Rolling leukocytes were defined as leukocytes that moved at a velocity slower than that of free-flowing leukocytes. The number of rolling leukocytes was calculated from the number of such leukocytes crossing a fixed area of the vessel per minute at a distance 1 disc diameter from the optic disc center. The definitive number of rolling leukocytes was defined as the total number of such leukocytes in all major veins. The velocity of rolling leukocytes was calculated as the time required for leukocytes to travel a given distance along the vessel.

The number of leukocytes that accumulated in the retinal microcirculation was evaluated at 30 min after AO injection. The number of fluorescent dots in the retina within 8–10 areas of 100 pixels square at a distance of 1 disc diameter from the edge of the optic disc was counted. The average number of individual areas was used as the number of leukocytes accumulated in the retinal microcirculation for each rat.

To monitor the venous wall shear rate in each retinal vein, we substituted the maximal velocity of flowing leukocytes (\(V_{wbc}\)) for the centerline red blood cell velocity (43). The mean red blood cell velocity (\(V\)) was estimated as \(V_{wbc}/1.6\). Venous wall pseudoshear rate was calculated based on the Newtonian definition: pseudoshear rate = \((V/D) \times 8 / s\), where \(D\) is the venular diameter.

After this experiment, the rats were killed with an anesthetic overdose, and the eyes were enucleated to determine a calibration factor with which to convert values measured on a computer monitor (in pixels) into real values (in \(\mu m\)).

Statistical Analysis

All values are presented as means ± SE. The data were analyzed by using an analysis of variance, with post hoc comparisons tested using Fisher’s protected least-significant difference procedure. Differences were considered statistically significant when the \(P\) values were <0.05.

RESULTS

Diameters of Major Retinal Vessels

Table 2 indicates the physiological variables in each group during the experiments. Figure 1, A–D, shows characteristic fundus images of a nonoperated control eye and postischemic eyes of nondiabetic rats at various time points after reperfusion. In arteries, significant vasoconstriction occurred immediately after reperfusion (65.5–77.9%, \(P < 0.01\) vs. control rats), with subsequent minor vasodilation in the nondiabetic group. Although postischemic arterial vasoconstriction...
was somewhat less in diabetic rats than in nondiabetic rats, there was no significant difference in the arterial diameter between those two groups (Fig. 1E).

In contrast to that in the artery, the postischemic change in venous diameter was biphasic. In nondiabetic rats, postischemic veins showed significant vasoconstriction at 4 h after reperfusion (85.0% of control values, \( P = 0.0085 \)). Thereafter, however, significant vasodilation occurred; this peaked at 24 h after reperfusion (115.2% of control values, \( P = 0.0079 \)) and subsided at 48 h after reperfusion. In diabetic rats, although the postischemic change in venous diameter was biphasic, postischemic vasoconstriction (93.5% of control values) and subsequent vasodilation (109.6% of control values) were slightly less than in nondiabetic rats (Fig. 1F).

**Leukocyte Rolling**

Immediately after intravenous administration of AO, leukocytes stained selectively among the circulating blood cells (Fig. 2A). No rolling leukocytes were observed in the major retinal veins of nonoperated control rats. In nondiabetic rats, a small number of leukocytes were observed rolling along the venous walls at 4 h after reperfusion; their number subsequently increased substantially and peaked at 12 h after reperfusion (Fig. 2B). Diabetic rats, however, showed no active leukocyte-endothelial cell interactions throughout the entire reperfusion period. In diabetic rats, leukocyte rolling was significantly suppressed compared with that in nondiabetic rats (\( P < 0.0001 \)) (Fig. 2C). The number of rolling leukocytes in diabetic rats was reduced by 73.6 (\( P = 0.0012 \)) and by 84.8% (\( P = 0.0019 \)) at 12 and 24 h after reperfusion, respectively, compared with nondiabetic rats.

The velocity of rolling leukocytes was significantly slower at 12 h after reperfusion in the nondiabetic group and at 4 h in the diabetic group, compared with values at other time points (Fig. 2D). On the basis of the entire reperfusion period, the velocity of rolling leukocytes in diabetic rats was significantly faster than that in nondiabetic rats (\( P < 0.0001 \)); that in diabetic rats was 148 (\( P = 0.0004 \)) and 147% (\( P = 0.0049 \)) of that in nondiabetic rats at 12 and 24 h after reperfusion, respectively.

The average venous wall pseudoshear rate in nondiabetic rats at 24 h after reperfusion was somewhat low compared with other measurements. Figure 3, A and B, shows the relationship between venous wall pseudoshear rate of each major retinal vein and the number of rolling leukocytes along it at 12 and 24 h after reperfusion. There was a weak correlation between the venous wall pseudoshear rate of each major retinal vein and the number of rolling leukocytes along it. However, the number of rolling leukocytes was suppressed in diabetic rats, regardless of the shear stress in each vein.

**Leukocyte Accumulation in Retinal Microcirculation**

At 30 min after AO injection, only accumulated leukocytes were recognized as distinct fluorescent dots (Fig. 4, A–D). In nondiabetic rats, few leukocytes were recognized in the control, whereas accumulated leukocytes began to increase with time after reperfusion and peaked at 24 h (Fig. 4E). In diabetic rats, the number of accumulated leukocytes in the preischemic period was about two times as many in diabetic rats as in nondiabetic rats. However, leukocyte accumulation during reperfusion in diabetic rats was significantly suppressed compared with nondiabetic rats (\( P < 0.0001 \)). The numbers of accumulated leukocytes were reduced by 31.1 (\( P = 0.026 \)) and 41.2% (\( P = 0.0018 \)) at 12 and 24 h, respectively, after reperfusion in diabetic rats compared with nondiabetic rats.

**Effects of Blood Glucose Levels on Leukocyte-Endothelial Cell Interactions**

Table 3 indicates the physiological variables during the experiments. Figure 5 shows the relationship between venous wall pseudoshear rate of each major retinal vein and the number of rolling leukocytes along it in each group. There was no significant difference in the venous wall pseudoshear rate in major retinal...
veins of nonischemic eyes between the nondiabetic (group 1) and diabetic (group 5) rats. However, ischemic eyes showed significant reduction in venous wall pseudoshear rate in all groups (groups 2–4 and 6–8), but there were no significant intergroup differences.

Figure 6 shows the number of rolling leukocytes along the major retinal veins and the number of leukocytes accumulated in the retina after reperfusion in each group. Values are means ± SE. *P < 0.01, †P < 0.05 compared with values in nondiabetic rats at each time point.

Leukocytes accumulated in the retina were observed as fluorescent dots at 30 min after acridine orange (AO) injection. A small number of leukocytes could be found in the control eyes of nondiabetic (A) and diabetic (B) rats. Increasing numbers of leukocytes accumulated after reperfusion and peaked at 24 h in a nondiabetic rat (C). Leukocyte accumulation was significantly suppressed in a diabetic rat at 24 h after reperfusion (D). Bar = 100 μm.

E: time course of the number of leukocytes accumulated in the retina after reperfusion in each group. In group 3, preischemic infusion of glucose to nondiabetic rats significantly raised the blood glucose level during ischemia induction. However, this treatment did not exacerbate leukocyte rolling or subsequent leukocyte accumulation during reperfusion. In addition, acute hyperglycemia induced by glucose infusion during the reperfusion
period had no significant influence on leukocyte-endothelial cell interactions in nondiabetic rats (group 4). In group 7, although preischemic insulin treatment to diabetic rats normalized the glucose level during ischemia induction, this treatment produced only minimal changes in leukocyte rolling and subsequent leukocyte accumulation. Furthermore, acute normoglycemia during reperfusion, induced with insulin treatment, had no substantial influence on the suppressed leukocyte-endothelial cell interactions in diabetic rats (group 8).

**DISCUSSION**

On the basis of clinical and experimental studies, a substantial amount of evidence indicates that diabetes or hyperglycemia at the onset of brain ischemia worsens the postischemic neurological outcome (14, 25). Recently, leukocytes have been suggested to contribute to this augmented ischemic damage in diabetic subjects (28). Experimental studies on leukocyte adhesion to endothelial cells have shown that leukocyte recruitment to postischemic tissue is mediated through a multistep process (6); each process is mediated by distinct adhesion molecules and regulated elaborately (2, 38). Therefore, investigation of leukocyte-endothelial cell interactions during reperfusion is essential to determine the role of leukocytes in posts ischemic neural damage in diabetics.

Recently, Panés et al. (28) demonstrated increased inflammatory leukocyte responses in diabetic mesentery during a 40-min reperfusion period after 10 min of ischemia. In contrast, in the present study, leukocyte-endothelial cell interactions in the postischemic retina were not augmented but, if anything, were suppressed in diabetic rats compared with nondiabetic rats. These conflicting findings could derive from a difference between somatic organs and the central nervous system and/or from the difference in the duration of the ischemia and reperfusion periods, which were much longer in our study. In the retina, 10-min ischemia induced no remarkable changes in leukocyte behavior during the reperfusion period (data not shown). Similarly, Dirmagl et al. (7) reported that leukocyte-endothelial cell interactions were only mildly active after 10 min of forebrain ischemia. In contrast, we previously reported that ischemia for 60 min resulted in activated leukocyte-endothelial cell interactions in postischemic retina and that leukocyte rolling and accumulation peaked at 12 and 24 h after reperfusion, respectively.

### Table 3. Physiological variables

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC, $\times 10^3$/μl</th>
<th>Blood glucose before ischemia, mg/dl</th>
<th>Blood glucose before AODF, mg/dl</th>
<th>MABP, mmHg</th>
<th>Heart rate, beats/min</th>
<th>Venous wall pseudoshear rate, $\times 10^3$/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>7.0 ± 0.7*†</td>
<td>137 ± 5†</td>
<td>491 ± 29*</td>
<td>107.8 ± 5.5</td>
<td>284 ± 13</td>
<td>1.62 ± 0.10</td>
</tr>
<tr>
<td>Group 2</td>
<td>9.4 ± 0.7</td>
<td>146 ± 8†</td>
<td>153 ± 12†</td>
<td>106.8 ± 4.2</td>
<td>292 ± 9</td>
<td>1.68 ± 0.07</td>
</tr>
<tr>
<td>Group 3</td>
<td>9.2 ± 0.8</td>
<td>146 ± 8†</td>
<td>153 ± 12†</td>
<td>103.8 ± 6.0</td>
<td>313 ± 8</td>
<td>1.84 ± 0.07</td>
</tr>
<tr>
<td>Group 4</td>
<td>8.3 ± 0.8</td>
<td>146 ± 8†</td>
<td>151 ± 28*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>5.6 ± 0.4*†</td>
<td>476 ± 15*</td>
<td>147 ± 14†</td>
<td>98.8 ± 0.9</td>
<td>291 ± 11</td>
<td>2.41 ± 0.1†</td>
</tr>
<tr>
<td>Group 6</td>
<td>9.9 ± 0.4</td>
<td>443 ± 14*</td>
<td>163 ± 11†</td>
<td>270 ± 14</td>
<td>1.62 ± 0.15</td>
<td>1.67 ± 0.11</td>
</tr>
<tr>
<td>Group 7</td>
<td>9.5 ± 0.5</td>
<td>503 ± 30*</td>
<td>163 ± 11†</td>
<td>301 ± 14</td>
<td>1.71 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Group 8</td>
<td>8.5 ± 1.1</td>
<td>105.2 ± 6.5</td>
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<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. AODF, acridine orange digital fluorography. *P < 0.01 compared with values in group 2. †P < 0.01 compared with values in group 6.

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Fig. 5. Relationship between venous wall pseudoshear rate of each major retinal vein and the number of rolling leukocytes along it at 12 h after reperfusion. ●, Values in the nondiabetic groups (1–4); ○, values in the diabetic groups (5–8).
Much histological evidence supports our observations, as Zhang et al. (44) demonstrated that neutrophil accumulation in rat cerebrum after 2 h of ischemia peaked at 24–48 h after reperfusion. Our observations are compatible with recent studies that showed that mRNA expression of adhesion molecules such as intercellular adhesion molecule-1 (38), E-selectin (45), and P-selectin (32) is upregulated after transient cerebral ischemia and peaks at 10–12, 12, and 8–24 h after reperfusion, respectively. Therefore, prolonged evaluation would be necessary to evaluate leukocyte-endothelial cell interactions in postischemic retina after transient ischemia.

In the present study, postischemic diabetic retina did not show active leukocyte-endothelial cell interactions throughout the reperfusion period. Diabetic animals are thought to have increased neural damage and worse neurological function after transient ischemia than nondiabetic animals (25). On the basis of results in the present study, leukocyte-mediated neural damage after transient cerebral ischemia would not be involved in this unfavorable outcome in diabetic animals. Accumulating evidence suggests that the higher glucose concentrations during ischemia lead to augmented anaerobic glycolytic product in the neural cells of diabetic brain (40). This exacerbated intracellular acidosis during ischemia in diabetic animals would result in greater neural damage (11, 29).

Unexpectedly, our study showed suppressed leukocyte-endothelial cell interactions in diabetic retina after transient ischemia. Although the mechanism for this suppressed leukocyte-endothelial interaction after transient ischemia is uncertain, the effect of adaptation would account for our findings. Diabetic organs are thought to be exposed to a relative chronic ischemia (21). Many experimental studies have indicated that retinal and cerebral blood flow are decreased during short periods of induced diabetes (23), whereas other studies have resulted in contradictory findings. Using a dye dilution technique, Bursell et al. (4) found that retinal blood flow is decreased by 37% as soon as 1 wk after induction of diabetes. It has been reported that chronic low-grade ischemia can cause a biochemical adaptation to severe ischemic insult (10). Tosaki et al. (34) reported that the diabetic heart would have a kind of ischemic preconditioning against an ischemic insult during a brief period of acute, uncontrolled diabetes. Intravital microscopic studies have shown that ischemic preconditioning reduces leukocyte-endothelial cell interactions during reperfusion (1, 19). Our hypothesis is supported by a previous direct vital microscopic study by Fortes et al. (12) that showed that diabetic animals have defective leukocyte-endothelial cell interactions under both basal and inflammatory conditions.

Similar to the findings in diabetic subjects, preischemic glucose infusion has also been reported to cause increased stroke volume and worse neurological function after cerebral ischemia (30). Moreover, preischemic insulin treatment to diabetic animals has been demonstrated to ameliorate the neural damage induced by transient cerebral ischemia (39). In the present study, however, neither preischemic insulin treatment to diabetic rats nor preischemic glucose infusion to nondiabetic rats had a significant influence on leukocyte-endothelial cell interactions in the postischemic retina. Therefore, although it is well known that intraischemic glucose concentrations have a close relationship to posts ischemic neural outcome (40), leukocyte-mediated neural damage after reperfusion could not account for this relationship. In addition, although a recent in vitro study has indicated that leukocyte-endothelial cell interactions are activated by high glucose concentrations (24), acute changes in glucose concentration during reperfusion had no marked effects in the present study. Taken together, acute changes in blood glucose concentrations either before or after ischemic induction do not exert a significant

Fig. 6. A: the flux of rolling leukocytes at 12 h after reperfusion in each group. B: the number of accumulated leukocytes at 12 h after reperfusion in each group. Values are means ± SE.*P < 0.01, †P < 0.05 compared with values in group 2, ‡P < 0.01, §P < 0.05 compared with values in group 6.
influence on postischemic leukocyte-endothelial cell interactions. Fortes et al. (12), who reported a defective leukocyte-endothelial cell interaction in diabetic rats, also indicated that its reversal was attained by daily insulin treatment for at least 12 days.

In conclusion, hyperglycemia at the onset of cerebral ischemia is thought to worsen postischemic neurologic outcome. In the present study, preischemic hyperglycemia induced by diabetes or glucose infusion did not augment leukocyte-endothelial cell interactions during reperfusion. Therefore, the unfavorable results after transient cerebral ischemia would not be due to leukocyte-mediated neural damage.

**Perspectives**

Acute changes of blood glucose concentrations before ischemic induction had no significant influence on leukocyte-endothelial interactions during the reperfusion period in this study. However, diabetic retinas showed suppressed leukocyte-endothelial interactions. Although the mechanism for this suppressed interaction is uncertain, the effect of adaptation would account for our findings. In nondiabetic rats, we have previously reported that P-selectin and intercellular adhesion molecule-1 play a central role in leukocyte-endothelial interactions after transient retinal ischemia. Suppressed expression of these adhesion molecules in the postischemic retina would contribute to suppressed leukocyte-endothelial interactions in diabetic postischemic retina. However, little information is available about the mechanism of adaptation of the diabetic rats to the ischemia.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

**REFERENCES**


