Microvascular Responses to Ischemia/Reperfusion in Normotensive and Hypertensive Rats

Iwao Kurose, Robert Wolf, Wolfgang Cerwinka, D. Neil Granger

Abstract—The objective of the present study was to determine whether long-term arterial hypertension renders the microvasculature more vulnerable to the deleterious inflammatory responses elicited by ischemia and reperfusion (I/R). Intravital fluorescence microscopy was used to monitor leukocyte adherence and emigration, platelet-leukocyte aggregation, and albumin extravasation in mesenteric postcapillary venules of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) after 10 minutes of ischemia and subsequent reperfusion. Significant and comparable increases in leukocyte adherence/emigration and the formation of platelet aggregates were elicited by I/R in both WKY and SHR. Albumin extravasation was enhanced after I/R in SHR, but not in WKY. Monoclonal antibodies directed against the adhesion glycoproteins CD18, P-selectin, or ICAM-1 showed similar patterns of protection against the I/R-induced inflammatory responses in WKY and SHR. The enhanced albumin extravasation noted in postischemic venules of SHR was prevented by immunoneutralization of either CD18 on leukocytes or ICAM-1 on endothelial cells. These results suggest that, whereas long-term arterial hypertension does not significantly modify the leukocyte and platelet recruitment normally elicited in venules by I/R, it does result in an exaggerated albumin leakage response, which is mediated by an interaction between \( \beta_2 \) (CD18) integrins on leukocytes and ICAM-1 on endothelial cells.

Key Words: leukocytes • integrins • oxidative stress • mast cells

Hypertension, diabetes, and hypercholesterolemia are known risk factors for the development of ischemic vascular disease. Although it is generally thought that these risk factors increase the incidence of ischemic diseases in the heart, brain, and gut by virtue of their ability to render organs more vulnerable to ischemic episodes (such as atherosclerosis-induced flow restriction in arterial vessels), mounting evidence shows that the risk factors, either alone or in combination, may also enhance the vulnerability of the microvasculature to the deleterious effects of ischemia and reperfusion (I/R). This enhanced vulnerability to I/R-induced microvascular dysfunction is often manifested as an amplification of the inflammatory cell-cell interactions, diminished endothelial barrier function, and enhanced oxidant production normally observed in postischemic venules.1–4 Hypercholesterolemia, for example, is associated with exaggerated leukocyte-endothelial cell adhesion, leukocyte-platelet aggregation, and oxidant production by endothelial cells in postcapillary venules subjected to I/R.4,5 In a similar manner, diabetes mellitus enhances the recruitment of inflammatory cells into postischemic microvessels, but this condition also results in an exacerbation of albumin extravasation across venules.

Although the effects of long-term arterial hypertension on the microvascular responses to I/R have not been characterized, evidence already in the literature supports the possibility that arterial hypertension may alter the vulnerability of the microvasculature to the deleterious actions of I/R. Indeed, published reports suggest that arterial hypertension could either enhance6,7 or attenuate8,9 the inflammatory responses elicited by inflammatory stimuli, such as I/R. The possibility of an enhanced I/R-induced inflammatory response in spontaneously hypertensive rats (SHR) is supported by reports that describe (1) an elevated blood leukocyte count and a greater proportion of basally activated granulocytes circulating in SHR versus Wistar-Kyoto rats (WKY)6 and (2) an increased spontaneous production of reactive oxygen metabolites in the vasculature of SHR10 and hypertensive Dahl salt-sensitive rats.11 Conversely, there are reports that describe an impaired leukocyte-endothelial cell adhesion in mesenteric venules of SHR (versus WKY) exposed to platelet activating factor (PAF), leukotriene \( \mathrm{B}_4 \) (LT\( \mathrm{B}_4 \)), or histamine,8,9 all of which have been implicated in the recruitment of leukocytes elicited by I/R.12 Furthermore, it has been shown recently that endotoxin-induced expression of ICAM-1, an endothelial cell adhesion molecule that has been implicated in I/R-induced leukocyte-endothelial cell adhesion,13 is significantly blunted in different vascular beds of SHR versus those of WKY.14

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Given the divergent inflammatory responses that have been observed in genetically hypertensive animals, it is difficult to predict whether and in what way this risk factor for ischemic vascular disease alters the microvascular responses to I/R. Hence, the major objective of this study was to determine whether long-term arterial hypertension renders the microvasculature more or less vulnerable to the deleterious inflammatory responses elicited by I/R. Because several leukocyte (CD11/CD18) and endothelial cell (ICAM-1, P-selectin) adhesion glycoproteins have been implicated in the altered immune responses of hypertension, we also examined the potential role of these adhesion receptors in mediating the microvascular responses to I/R in both normotensive and hypertensive animals. These objectives were addressed by applying the technique of intravital fluorescence microscopy to venules of SHR and their normotensive WKY controls.

The studies were performed with a rat mesentery model of I/R, which allows for simultaneous measurement of leukocyte-endothelial cell adhesion, platelet-leukocyte aggregation, and albumin extravasation. The findings of the present study indicate that long-term arterial hypertension does not significantly modify the leukocyte and platelet recruitment normally elicited in venules by I/R. However, this risk factor does result in an exaggerated albumin leakage response, which is mediated by an interaction between β2 (CD11B) integrins on leukocytes and ICAM-1 on endothelial cells.

### Methods

#### Surgical Procedure

Surgical procedures on animals used in these experiments were in accordance with guidelines of Louisiana State University. In this study, 30 male SHR and 30 normotensive control WKY were used. The animals were obtained from Harlan-Sprague Dawley (Frederick, Md) and weighed 250 to 325 g. After the animals were anesthetized with pentobarbital (65 mg/kg body weight), a tracheotomy was performed to facilitate breathing during the experiment. The right carotid artery was cannulated and systemic arterial pressure was measured with a pressure transducer (Statham P23A) connected to the carotid artery cannula. Systemic blood pressure and heart rate were continuously recorded with a physiological recorder (Grass Instruments). The left jugular vein and superior mesenteric artery were also cannulated for drug administration.

#### Intravital Microscopy

The methods and techniques used to monitor microvascular and inflammatory responses in mesenteric postcapillary venules of WKY and SHR were identical to those previously described. In brief, intravital microscopy was used to monitor leukocyte adherence and emigration, platelet-leukocyte aggregation, leakage of FITC-labeled bovine albumin (Sigma Chemical Co), red blood cell (RBC) velocity, and vessel diameter in mesenteric venules. Wall shear rate was calculated on the basis of the newtonian definition: $\gamma = 8(V_{\text{mean}}/D)$.

#### Experimental Protocols

After all parameters measured online were in a steady state, images from the mesenteric preparation were recorded on videotape for 10 minutes. Immediately thereafter, the superior mesenteric artery was ligated with a snare created from polyethylene tubing. On the basis of findings from a previous study, an ischemic period of 10 minutes (or 0 minutes for sham-operated controls) was used to elicit the acute inflammatory responses in both WKY and SHR. Rep perfusion was induced by gently removing the arterial ligature. All measured variables were recorded at 10-minute intervals for 30 minutes after the onset of reperfusion. In some experiments, the SHR and WKY (n=5 per group) were pretreated (15 minutes before control measurements) with a monoclonal antibody (mAb) directed against either CD18 (CL26, 100 μg/rat), ICAM-1 (1A29, 2 mg/kg), P-selectin (PB1.3, 2 mg/kg), or a nonbinding antibody (P6H6, 2 mg/kg). The same protocol was used with a 10-minute ischemic period. CL26 and 1A29 were provided by Pharmacia-UpJohn Laboratories, whereas PB1.3 and P6H6 were provided by Cytel Corporation. The blocking dose used for each mAb was determined from previously published studies. At the doses used, none of the mAbs caused leukopenia. The microvascular and inflammatory responses to these treatment regimens in WKY and SHR were compared with the responses of animals that did not receive any treatment.

#### Statistics

The data were analyzed by use of standard statistical analysis; 1-way ANOVA and the Fisher exact (post hoc) test. All values are reported as mean ± SE from 5 to 7 rats, and statistical significance was set at $P<0.05$.

#### Results

Table 1 summarizes venular diameter, RBC velocity, and wall shear rate data obtained in WKY and SHR. The mean arterial pressure in SHR (145 ± 9 mm Hg) was significantly higher than in WKY (101 ± 4 mm Hg). The control values refer to sham-operated rats exposed to the same procedures without ischemia. Ten minutes of complete ischemia followed by 30 minutes of reperfusion resulted in comparable reductions in both RBC velocity and wall shear rate in WKY and SHR. These I/R-induced changes in RBC velocity and shear rate in WKY and SHR were not affected by treatment with mAbs directed against CD18, ICAM-1, P-selectin, or a nonbinding mAb.

Figure 1 illustrates the effects of I/R on the number of adherent leukocytes in mesenteric venules of WKY and SHR. It shows how the different adhesion glycoprotein-directed mAbs affect the I/R-induced leukocyte adherence responses.

### Table 1. Effects of I/R on Venular Diameter, RBC Velocity, and Wall Shear Rate in SHR and WKY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diameter, μm</th>
<th>RBC Velocity, mm/sec</th>
<th>Wall Shear Rate, 1/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>30.8 ± 1.1</td>
<td>3.36 ± 0.17</td>
<td>561 ± 12</td>
</tr>
<tr>
<td>I/R</td>
<td>30.0 ± 1.2</td>
<td>2.50 ± 0.25*</td>
<td>415 ± 35†</td>
</tr>
<tr>
<td>+CD18 mAb</td>
<td>31.0 ± 1.3</td>
<td>2.64 ± 0.18</td>
<td>427 ± 26</td>
</tr>
<tr>
<td>+ICAM-1 mAb</td>
<td>30.4 ± 0.8</td>
<td>2.36 ± 0.17</td>
<td>390 ± 28</td>
</tr>
<tr>
<td>+P-selectin mAb</td>
<td>29.6 ± 1.3</td>
<td>2.34 ± 0.24</td>
<td>395 ± 35</td>
</tr>
<tr>
<td>+NB mAb</td>
<td>30.8 ± 1.3</td>
<td>2.44 ± 0.19</td>
<td>396 ± 27</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>30.2 ± 1.8</td>
<td>3.68 ± 0.14</td>
<td>613 ± 16</td>
</tr>
<tr>
<td>I/R</td>
<td>30.0 ± 1.3</td>
<td>2.52 ± 0.25*</td>
<td>418 ± 29†</td>
</tr>
<tr>
<td>+CD18 mAb</td>
<td>30.4 ± 0.8</td>
<td>2.50 ± 0.19</td>
<td>411 ± 29</td>
</tr>
<tr>
<td>+ICAM-1 mAb</td>
<td>30.0 ± 1.5</td>
<td>2.46 ± 0.18</td>
<td>413 ± 28</td>
</tr>
<tr>
<td>+P-selectin mAb</td>
<td>30.0 ± 1.2</td>
<td>2.18 ± 0.24</td>
<td>360 ± 27</td>
</tr>
<tr>
<td>+NB mAb</td>
<td>30.2 ± 1.3</td>
<td>2.42 ± 0.14</td>
<td>400 ± 18</td>
</tr>
</tbody>
</table>

Data are presented at 30 minutes after reperfusion. NB mAb indicates nonbinding mAb. $P<0.05$ vs corresponding control value. Each experimental group consisted of 5 animals.
In both WKY and SHR groups, I/R elicited a significant and comparable increase in the number of firmly adherent leukocytes. In WKY, mAbs directed against CD18, ICAM-1, and P-selectin resulted in an attenuation of leukocyte adherence by 57%, 49%, and 41%, respectively, whereas the nonbinding mAbs had no effect. A similar pattern of effectiveness of the mAbs in reducing I/R-induced leukocyte adherence was noted in SHR. As shown in Figure 2, the responses of leukocyte emigration to I/R in WKY and SHR largely paralleled the changes noted for leukocyte adherence. A difference between the leukocyte adherence and emigration responses was the inability of the P-selectin specific mAb to reduce I/R-induced leukocyte emigration in both WKY and SHR.

Figure 3 summarizes the responses of albumin leakage in mesenteric venules of WKY and SHR to I/R and illustrates how the different adhesion glycoprotein-directed mAbs affect the I/R-induced changes in albumin leakage. A difference between WKY and SHR was the inability of I/R to elicit a significant increase in albumin leakage in WKY, although a profound increase was observed in mesenteric venules of SHR. The enhanced I/R-induced albumin leakage noted in SHR was largely prevented by mAbs directed against CD18 or ICAM-1, but not by P-selectin or a nonbinding mAb.

Exposure of the rat mesentery to I/R is associated with the formation of large platelet-leukocyte aggregates that fill the venule lumen and rapidly course through the vessel with flowing blood. Although such aggregates are never observed during control conditions, 37.4 ± 8.4% (in SHR) and 28.5 ± 9.4% (in WKY) of the luminal area of postcapillary venules was occupied by platelet-leukocyte aggregates after exposure to 10 minutes of ischemia and 30 minutes of reperfusion. Table 2 summarizes the effects of the different mAbs on I/R-induced formation of platelet-leukocyte aggregates. Although the most profound reduction in aggregate formation was observed in WKY (84%) and SHR (67%) that received the P-selectin mAb, significant reductions in aggregate formation were also noted in animals treated with an mAb directed against either CD18 (SHR, 73%; WKY, 61%) or ICAM-1 (84%; SHR, 67%).

### Table 2. Effects of I/R on Platelet-Leukocyte Aggregation in SHR and WKY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Luminal area, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>I/R</td>
<td>28.5 ± 9.4*</td>
</tr>
<tr>
<td>+ CD18 mAb</td>
<td>11.0 ± 5.8†</td>
</tr>
<tr>
<td>+ ICAM-1 mAb</td>
<td>11.4 ± 4.1†</td>
</tr>
<tr>
<td>+ P-selectin mAb</td>
<td>9.6 ± 4.2†</td>
</tr>
<tr>
<td>+ NB mAb</td>
<td>25.9 ± 9.0</td>
</tr>
</tbody>
</table>

Values refer to luminal area occupied by platelet-leukocyte aggregates at 30 minutes after reperfusion. NB mAb indicates nonbinding mAb.

*P < 0.05 vs corresponding control value.
† Each experimental group consisted of 5 animals.
or ICAM-1 (SHR, 71%; WKY, 60%), but not in those treated with the nonbinding mAb.

Discussion

It is well recognized that the microvasculature is particularly vulnerable to the deleterious effects of I/R. The I/R-induced microvascular dysfunction is manifested as a diminished endothelium-dependent vasodilatation in arterioles,19,20 enhanced fluid filtration in capillaries,21 and a disrupted endothelial barrier (increased permeability) in postcapillary venules.12 The responses observed in all segments of the microvasculature appear to be elicited, at least in part, by activated and adherent leukocytes, which are recruited to postischemic tissues as a result of an enhanced, oxidant-mediated generation of inflammatory agents (such as PAF and LTB4). Xanthine oxidase and adherent leukocytes represent major sources of the oxidants that initiate the cascade of events that lead to the leukocyte-endothelial cell adhesion and endothelial dysfunction associated with I/R.12 Whereas the mechanisms underlying the microvascular responses to I/R have been extensively studied in otherwise normal animals, relatively little is known about how the well-established risk factors for cardiovascular disease influence the responses of the microvasculature to I/R. Studies performed on diabetic1 and hypercholesterolemic4 animals suggest that these risk factors profoundly exacerbate the inflammatory cell recruitment and microvessel dysfunction induced by I/R. However, the effects of long-term arterial hypertension on the microvascular responses to I/R have not yet been systematically addressed, despite the existence of a large body of evidence indicating that genetically hypertensive rats exhibit significantly altered immune responses.6–11

The major objective of this study was to determine whether long-term arterial hypertension renders the microvasculature more or less vulnerable to the deleterious inflammatory responses elicited by I/R. By use of SHR and their normotensive counterparts WKY, we compared the ability of postcapillary venules in these animals to mount an inflammatory response after challenge with I/R. Our findings indicate that the microvascular alterations normally elicited by I/R do not differ significantly between SHR and WKY with 1 notable exception, albumin extravasation. The number of firmly adherent and emigrated leukocytes and the amount of platelet-leukocyte aggregation in postischemic venules of SHR were quite similar to those noted in WKY. However, whereas albumin leakage from postcapillary venules was unaffected by 10 minutes of ischemia and 30 minutes of reperfusion in WKY, a dramatic increase in albumin extravasation was noted in SHR. Our experiments with adhesion molecule–specific mAbs also revealed that the I/R-induced albumin leakage across venules in SHR is mediated by an adhesive interaction between CD11/CD18 on leukocytes and ICAM-1 on endothelial cells.

Our finding that the I/R-induced leukocyte recruitment responses in SHR are similar to those noted in WKY contrasts with reports describing an attenuated recruitment of adherent leukocytes in mesenteric venules of SHR (relative to WKY) that are exposed to inflammatory mediators such as PAF, LTB4, and histamine.8,9 Antagonists and biosynthesis inhibitors that target these mediators have been shown to blunt I/R-induced leukocyte-endothelial cell adhesion in mesenteric venules of normotensive rats.12 Hence, a comparable attenuation of leukocyte adhesion in SHR would be expected after exposure of venules to I/R.

However, published evidence suggests that I/R should elicit an exacerbation of leukocyte adhesion in venules of SHR versus WKY. For example, it has been shown that a large number of circulating leukocytes in SHR (versus WKY) are in an activated state, as reflected by an enhanced rate of superoxide production.6 Furthermore, there is evidence that the constitutive (basal) level of ICAM-1 expression is significantly higher in the splanchnic microvasculature of SHR than in WKY.14 Finally, a recent report describes an enhanced adhesion of isolated monocytes to aortic endothelial cells from SHR versus WKY.22 An explanation for the inconsistency between these observations and the reports describing a reduced sensitivity of venules in SHR to PAF, LTB4, or histamine6,9 is not readily available. However, it may reflect the involvement of different mechanisms for I/R-induced recruitment of adherent leukocytes in WKY versus SHR.

A notable difference in the responses of the microvasculature of SHR versus WKY to I/R is the significantly greater albumin leakage that occurs in SHR. This observation is interesting in view of published reports that describe either a diminished23 or unchanged24 endothelial barrier function in hypertensive humans or SHR. Consistent with some reports of a diminished endothelial barrier function, previous studies of I/R-induced albumin leakage in normotensive animals have used longer ischemic durations to elicit the level of endothelial barrier dysfunction that we observed in venules of SHR. Nonetheless, previous studies have established a clear cause and effect relationship between I/R-induced albumin leakage and the adhesion and emigration of leukocytes in postcapillary venules.13 Indeed, it has been shown that the magnitude of the albumin extravasation in mesenteric venules elicited by I/R is directly proportional to the number of adherent and emigrated leukocytes.13 Furthermore, mAbs that blunt the recruitment of adherent leukocytes after I/R are similarly effective in reducing the accompanying albumin leakage response. In that regard, it is interesting that the findings of the present study demonstrate that, although the intensity of I/R-induced leukocyte recruitment in venules of SHR is no greater than that observed in WKY, the endothelial barrier in venules of SHR appears to be more vulnerable to the leukocyte-mediated damage induced by I/R. This enhanced vulnerability may reflect an enhanced capacity of leukocytes to mediate tissue injury in SHR, because it has been shown that neutrophils from SHR degranulate more readily and produce more superoxide when stimulated than their counterparts do in WKY.6,7 Alternatively, it may be proposed that vascular endothelial cells in SHR produce less NO than those in WKY, and, as a consequence, SHR venules have a reduced capacity to defend against neutrophil-derived superoxide. However, this possibility appears unlikely in view of a previous report that describes nearly identical leukocyte-endothelial cell adhesion responses after NO synthase inhibition in mesenteric venules of SHR and WKY.8
In conclusion, our findings suggest that long-term arterial hypertension does not exacerbate the recruitment of leukocytes into postischemic tissues to the same degree as other cardiovascular risk factors, such as diabetes and hypercholesterolemia. However, hypertension does appear to render the endothelial barrier more susceptible to permeability. This increases the effect of I/R, an action that is mediated by an interaction between CD11/CD18 on leukocytes and ICAM-1 on endothelial cells.

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References

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