Fucoidin Reduces Coronary Microvascular Leukocyte Accumulation Early in Reperfusion

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Background. Leukocytes rapidly accumulate in the heart early in reperfusion after ischemia, contributing to reperfusion injury. The purpose of this study was to determine whether treatment with the selectin blocker fucoidin (FCN) would attenuate early leukocyte retention in coronary venules and capillaries during low flow reperfusion.

Methods. Isolated rat hearts subjected to 30 minutes of 37°C, no-flow ischemia were initially reperfused with blood containing labeled leukocytes, followed by reperfusion with a Krebs red cell solution. The deposition of leukocytes in coronary capillaries and venules was observed using intravital microscopy. Three groups were studied: nonischemic control hearts, untreated postischemic hearts reperfused at low flow, and postischemic hearts reperfused at low flow, where both the hearts and the blood reperfusate were pretreated with FCN (0.36 mg/mL blood).

Results. In the ischemia-reperfusion group, we observed a rapid and significant increase in leukocyte accumulation in both capillaries and venules. Treatment with FCN significantly reduced the leukocyte accumulation in both capillaries and venules (p < 0.05). In addition, FCN significantly reduced the persistence of leukostasis in both capillaries and venules, indicating that FCN affected a transient adhesion process.

Conclusions. These results suggest that the selectin family of leukocyte–endothelial cell adhesion proteins mediates the initial retention of leukocytes in both coronary capillaries and venules during reperfusion. Selectin blockade may be effective in reducing the contribution of leukocytes to early reperfusion injury.

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vious studies indicate that the rapid accumulation of leukocytes significantly impairs the initial recovery of the heart from ischemia [2, 3]. In addition, experimental evidence indicates that leukocytes accumulate in the microcirculation of the heart during the first minutes of reperfusion [6] and that this accumulation is greater when blood flow is reduced [7, 11].

Fucoidin (FCN) is a complex polysaccharide derived from seaweed and acts as a nonspecific blocker of P- and L-selectin [12]. In the mesentery, FCN treatment has been reported to decrease leukocyte rolling to vascular endothelium during ischemia and early reperfusion [13]. In a recent study [5] in the heart, FCN treatment improved left ventricular function during early reperfusion after cold ischemia. However, the effects of FCN on leukocyte accumulation in the coronary microcirculation during the first minutes of reperfusion have not been examined.

Therefore, the purpose of these experiments was to determine whether selectin blockade with FCN, a non-selective selectin blocker, would attenuate the initial leukocyte sequestration in the coronary microcirculation during low flow reperfusion after ischemia. We found that FCN treatment did significantly decrease leukocyte sequestration in the coronary microcirculation. These results suggest that during reperfusion after myocardial ischemia, P- and L-selectin may mediate the initial leukocyte accumulation in capillaries and venules, and thus contribute to leukocyte-mediated early reperfusion injury.

Material and Methods

Isolated Rat Heart Preparation

All animal experiments in this study were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH publication 85-23, revised 1985). A modified Langendorff preparation was used to perfuse the heart and visualize the coronary microcirculation (Fig 1) and has been described in detail previously [6, 7, 14, 15]. Adult male Sprague-Dawley rats (400 to 600 g) were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg) and then tracheotomized. Each animal was resuscitated (Harvard, model 683) and a median sternotomy was performed to visualize the great vessels of the heart. Loose ligatures were placed around the right innominate artery and ascending aorta. Heparin (150 U) was then injected into the right atrium. Ligatures were placed and tied around the right subclavian and common carotid arteries. A catheter (no. 20 Jelco) was then inserted into the innominate artery, advanced until the tip extended just into the aorta, and secured. A small hole was cut in the right atrium and a previously placed aortic ligature was quickly tied. The heart was initially arrested with a cold cardioplegia solution. The arresting solution was manually infused at a maximum rate of 3 mL/min and at a pressure less than 80 mm Hg. The heart was then carefully removed from the thoracic cavity, placed on a special Lucite stage, and connected to the perfusion circuit. The heart was covered with premoistened gauze in preparation for intravital fluorescence microscopic evaluation of the left ventricular epicardial microcirculation.

Preparation of Coronary Perfusate

The coronary perfusate used in this study was a modified Krebs–bicarbonate solution (NaCl 90 mmol/L, KCl 30 mmol/L, CaCl2 2.5 mmol/L, KH2PO4 1.2 mmol/L, MgSO4·7H2O 1.2 mmol/L, NaHCO3 25 mmol/L, glucose 5 mmol/L, EDTA 0.08 mmol/L) that contained 2.0 g/100 mL bovine serum albumin (fraction V, Sigma) and washed human red cells (American Red Cross) to a hematocrit of 20% [6, 7, 14, 15]. The red cell-containing perfusate is referred to as K2RBC. To maintain a stationary field for visualizing the coronary microcirculation, the potassium in the perfusate was increased to 30 mmol/L. The sodium was reduced to maintain isotonicity. The K2RBC contains virtually no leukocytes or platelets. The perfusate was gassed with 95% O2–5% CO2 and pH was corrected by adding 8.4% sodium bicarbonate. Blood gases were measured throughout the experiment with a blood gas analyzer (Radiometer, ABL 330). The use of a red cell-rich Krebs–albumin perfusate preserves vascular tone and integrity and provides superior myocardial oxygenation than Krebs solution alone. With
the use of K(2)RBC, coronary blood flows closely approximate those observed in the rat in vivo [14].

**Preparation of Labeled Diluted Whole Blood**

The procedure for labeling the leukocytes in diluted whole blood was described previously [6, 7, 15]. Briefly, freshly drawn heparinized blood was obtained by cardiac puncture from an anesthetized donor rat. The blood was separated by gentle centrifugation (Baxter, Megafuge 1.0R). The white cell pellet was incubated in 4 mL of an acridine orange solution (0.01 mg acridine orange solution/mL phosphate-buffered saline) (Sigma), incubated at room temperature for 15 minutes, then washed twice with a phosphate-buffered saline/albumin solution. The labeled cells were recombined with the red blood cells and plasma and then diluted 1:1 with modified Krebs. The solution was referred to as labeled diluted whole blood (DWB*). DWB* was warmed in a 37°C water bath (Precision, model 181) for 10 minutes before perfusion. This method of preparing diluted whole blood does not activate neutrophils [6, 16].

**Direct Visualization of the Coronary Microcirculation**

During reperfusion, the coronary epicardial microcirculation was directly visualized with a fluorescence microscope (Zeiss, MPS). The microcirculation was initially brought into focus using a 5x objective. A 32x objective was then used to collect data. With the 32x objective, the specimen to monitor magnification was 780x. Images were recorded periodically on a ½-in. videotape recorder (Mitsubishi, U82). At each observation point, five to seven coronary capillary fields and five to seven venules (20 to 100 μm in diameter) were selected at random and videotaped for at least 30 seconds. On video playback, the number of leukocytes that were in the plane of focus and remained stationary in a capillary for at least 30 seconds were considered trapped. These cells were counted and expressed as leukocytes/capillary field. For the venules, the leukocytes that were in the plane of focus on the top and bottom margins of the vessel and remained stationary for at least 30 seconds were counted as adhered and expressed as leukocytes/100 mm venule [6, 7, 15]. This measure is representative of leukocyte adhesion to venules.

**Measurement of Shear Rates**

Measurement of shear rate requires a measurement of microvascular blood velocity and vessel diameter. The centerline velocity of WBCs ($V_{WBC}$) was measured using the labeled leukocytes as natural markers of blood flow [7]. The $V_{WBC}$ was determined in 40- to 70-μm venules during the first 7 minutes of reperfusion, when blood containing fluorescently labeled leukocytes was delivered to the heart. During video playback, $V_{WBC}$ (in μm/s) was measured as the distance the leading edge of a leukocyte traveled in three or more video frames. The $V_{WBC}$ was calculated as 30 frames/s × distance traveled (μm) per number of frames. For each venule, three to six $V_{WBC}$ were measured and averaged. Shear rate was subsequently calculated based on Poiseuille flow as: $8(V_{WBC}/D)$, where $V_{WBC} =$ centerline velocity/1.6 [7].

**Other Physiological Measures**

The coronary circulation was perfused at constant flow at 3 mL/min (full flow) or 0.3 mL/min (low flow). The flow was confirmed periodically by a timed collection of perfusate into a graduated cylinder. Coronary perfusion pressure was measured continuously through a sidetube with a pressure transducer. Coronary vascular resistance was calculated as coronary perfusion pressure (mm Hg) per coronary flow (in mL/min) per heart weight (in grams).

**Experimental Protocol**

Figure 1 is a diagram of the isolated heart preparation used to study the coronary microcirculation (top) and a diagram of the experimental protocol (bottom). The postischemic low reflow model was developed subsequent to our initial observations that under conditions of full flow reperfusion with unactivated blood there was a significant increase in leukocyte trapping in capillaries but not in venules [6]. Using the low reflow model, we demonstrated that a significant reduction in blood shear rate resulted in a both significant leukocyte adhesion to postischemic coronary venules and a further increase in leukocyte trapping in coronary capillaries [7]. Therefore, in the current study we wanted to examine the effects of selectin blockade on leukocyte retention in venules and capillaries during low flow reperfusion. Three groups were studied. A nonischemic control group (NIC, n = 9 hearts), an untreated group subjected to ischemia and reperfused at 10% of full flow (I-R, n = 7 hearts) with DWB* pretreated with a vehicle (phosphate-buffered saline), and a third group in which hearts were treated with FCN (0.36 mg/mL blood) [13, 17] for 5 minutes before ischemia, and reperfused at 10% of normal flow with blood that was incubated with FCN (0.36 mg/mL blood) for 20 minutes before reperfusion (I-R FCN, n = 8). Nonischemic control hearts were perfused at 3 mL/min for 60 minutes with K(2)RBC, then for 7 minutes with DWB* then with K(2)RBC at 3 mL/min. Hearts subjected to ischemia were initially perfused at 3 mL/min for 30 minutes at 36°C to 37°C with K(2)RBC. After this 30-minute preischemic period, the hearts were subjected to 30 minutes of normothermic (36°C to 37°C) global ischemia. Postischemic hearts were reperfused for 7 minutes with DWB* at 10% of full flow (0.3 mL/min) followed by K(2)RBC at 0.3 mL/min. The total reperfusion period was 35 minutes. Heart temperature was recorded continuously with a pediatric temperature probe and monitor (YSI). DWB* was perfused with a syringe pump (Harvard Apparatus, model 11) through a side port, located immediately upstream of the heart (Fig 1). During reperfusion, microvascular fields were videotaped at 5 (R5), 20 (R20), and 35 minutes (R35) to assess leukocyte retention. Microvascular fields were videotaped at corresponding time
points in control hearts. White blood cell velocities were also measured during video playback.

**Statistical Analysis**

Data were collected and tabulated on computer spreadsheets (Microsoft Excel 5.0). Summary data were expressed as means ± standard errors. Comparisons among groups were made by analysis of variance. A Scheffé post hoc analysis was performed when appropriate (Statistica 3.1, Statsoft). Also, the RS leukocyte accumulation data was analyzed using the Kruskall-Wallis test. Probabilities of 0.05 or less were considered statistically significant.

**Results**

**Leukocyte Accumulation in the Coronary Capillaries**

Figure 2 shows representative images of leukocyte accumulation in coronary capillaries (left images) after 5 minutes of reperfusion in the three groups studied. Few leukocytes were retained in the capillaries of nonischemic control hearts (A). Low flow reperfusion resulted in a significant increase in leukocyte accumulation in the capillaries (C). In addition to single leukocytes trapped in capillaries, rows of trapped leukocytes within the same capillary was a common observation during low flow reperfusion. When FCN-treated hearts were reperfused...
at 10% of normal flow (E), there was a modest decrease in the number of leukocytes retained in the capillaries. Figure 3 is a summary of the number of leukocytes retained in coronary capillaries at R5, R20, and R35 in the three groups studied. When reperfusion was reduced to 10% of control, leukocyte retention at R5 in the capillaries was almost five times greater than nonischemic control values (R5: NIC = 2.0 ± 0.3 leukocytes/capillary field versus I-R = 9.2 ± 0.8 leukocytes/capillary field, p < 0.05). In the group pretreated with FCN, leukocyte retention in capillaries was significantly decreased compared with the I-R group (R5: I-R FCN = 5.0 ± 0.7 leukocytes/capillary field, p < 0.05). Treatment did not completely eliminate leukocyte trapping in capillaries because this value remained significantly greater than the nonischemic control values (p < 0.05). At R20 and R35, leukocyte retention in the I-R group remained about four to seven times greater than control values (p < 0.05). At these time points, the number of leukocytes remaining in the capillaries of the FCN-treated group was significantly less than the I-R values (p < 0.05).

The persistence of leukocyte retention in the coronary capillaries is as follows: NIC, 39%; I-R, 57%; and I-R FCN, 28%. Persistence is a measure of the enduring nature of the initial leukocyte retention in capillaries and is expressed as a percentage of R35 video count/R5 video count values. The percentage of leukocytes retained in the I-R group (57%) was approximately one and a half times greater than the NIC group (39%). Fewer leukocytes were retained in the capillaries in postischemic group treated with FCN (28%).

Leukocyte Accumulation in Coronary Venules

The images in Figure 2, right, are representative of leukocyte accumulation in coronary venules after 5 minutes of reperfusion. We rarely observed leukocytes adhering to the venules of nonischemic hearts. Compared to control hearts (B), there was a significant increase in leukocyte adhesion in venules of postischemic hearts reperfused at 10% of normal flow (D). In the postischemic group pretreated with FCN, we observed a significant reduction in the number of leukocytes adhered to the venules (F).

The average number of leukocytes adhered to the coronary venules during reperfusion is summarized in Figure 4. At R5, there was a statistically significant, threefold increase in leukocyte adhesion to venules of postischemic hearts reperfused at 10% of normal flow compared with nonischemic controls (R5: NIC = 1.5 ± 0.5 leukocytes/100 μm venule versus I-R = 4.4 ± 0.5 leukocytes/100 μm venule, p < 0.05). When the ischemic hearts and the blood reperfusion were pretreated with FCN and reperfused at 10% of normal flow, leukocyte adhesion to venules was decreased to control values (R5: I-R FCN = 1.8 ± 0.3 leukocytes/100 μm venule, p < 0.05 versus I-R). In contrast to the persistence of leukocytes trapped in capillaries, there was a dramatic decrease in the number of leukocytes remaining in the venules of all groups after 35 minutes of reperfusion (NIC, 6%; I-R, 10%; I-R FCN, 6%). With respect to leukocyte persistence, more leukocytes remained adhered to the venules of nontreated, postischemic hearts reperfused at low flow (10%) compared with nonischemic controls (6%). The
Average venular diameter was 60 μm in treated; NIC remained greater than control values. Less than (coronary resistance values in the I-R FCN group were greater than NIC values. At R35, reperfusion, coronary resistance values in the I-R and I-R groups during the preischemic period (Fig 5). During video playback in 35- to 70-μm venules (Table 1). Average venular diameter was 60 μm. In the NIC group, when blood flow was 3 mL/min, some leukocytes traveled across the entire field (340 μm) and out of view in one video frame, representing a velocity of 10,500 μm/s or greater. In the NIC group, centerline WBC velocity was greater than 2,760 ± 279 μm/s and estimated shear rate was greater than 301 ± 30 s⁻¹. In the I-R group, velocity and shear rate were significantly reduced to 864 ± 90 μm/s and 84 ± 10 s⁻¹, respectively. The V_{WBC} and shear rate in the I-R FCN group were not significantly different than the I-R group (1,032 ± 162 μm/s and 106 ± 19 s⁻¹, respectively).

**Coronary Vascular Resistance**

Coronary vascular resistance (mm Hg · mL⁻¹ · min⁻¹ · g⁻¹) was not significantly different among the three groups during the preischemic period (Fig 5). During reperfusion, coronary resistance values in the I-R and I-R FCN groups were greater than NIC values. At R35, coronary resistance values in the I-R FCN group were less than (p = not significant) the I-R group, but remained greater than control values.

**Comment**

Reperfusion injury is associated with a series of events that detract from the overall benefit of restoration of blood flow. However, the benefits of returning blood flow to an ischemic organ clearly outweigh any additional injury caused by reperfusion. Efforts to minimize the complications of reperfusion are warranted, especially in light of the ever-increasing number of surgical and medical cases in which reperfusion occurs (eg, revascularization, transplantation, thrombolysis, and angioplasty). A large body of evidence indicates that leukocytes contribute to I-R injury. In the heart, during reperfusion, leukocytes mediate additional myocyte and vascular damage by accumulating in the coronary microcirculation. White blood cells physically obstruct coronary capillaries and contribute to no-reflow [4]. Activated WBCs release oxygen free radicals, and later proteolytic enzymes, thromboxanes, and leukotrienes [18]. There are both early (within minutes) and late (within hours) components to leukocyte-mediated reperfusion injury and the mechanisms underlying early and late injury may be different. Early leukocyte-mediated reperfusion injury is likely initiated while the leukocytes are still intravascular, whereas the later injury is associated with leukocyte migration from the vasculature. Early injury can include a significant decrease in the recovery of left ventricular pump function [3, 19].

Leukocyte sequestration in the coronary microcirculation is one of the first steps in the inflammatory reaction initiated by myocardial ischemia and reperfusion. The exact locations and the mechanisms of leukocyte accumulation in the microcirculation during the first minutes of reperfusion have been examined previously [6]. The extent of leukocyte accumulation depends on the level of blood activation and the blood cell–blood vessel shear forces during reperfusion. We previously found that when postschismic hearts were reperfused under normal flow conditions with unactivated blood, leukocytes accumulated primarily in coronary capillaries and, to a lesser extent, in coronary venules. When the WBCs were either preactivated [6] or when blood flow was severely reduced [7], a statistically significant increase in leukocyte accumulation in coronary venules was observed. These findings also suggest that leukocyte accumulation in the coronary microcirculation occurs immediately with reperfusion. There is evidence to suggest that intravascular leukocyte accumulation during the first minutes of reperfusion may be sufficient to initiate tissue injury. Data from this laboratory [2, 3] and others [20, 21] demonstrate that reperfusion with blood for only 5 minutes impairs the recovery of left ventricular function and causes microvascular dysfunction.

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**Table 1. White Blood Cell Velocities and Shear Rates in the Coronary Microcirculation in the Nonischemia Control, Ischemia–Reperfusion, and Ischemia–Reperfusion, Fucoidin Treated Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Vessels</th>
<th>Velocity (μm/s)</th>
<th>Shear Rate (s⁻¹)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIC</td>
<td>38</td>
<td>&gt;2,760 ± 279</td>
<td>&gt;301 ± 30</td>
</tr>
<tr>
<td>I-R</td>
<td>35</td>
<td>864 ± 90</td>
<td>84 ± 10⁴</td>
</tr>
<tr>
<td>I-R FCN</td>
<td>33</td>
<td>1,032 ± 162</td>
<td>106 ± 19⁴</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with nonischemic control.

IR = ischemia–reperfusion; I-R FCN = ischemia–reperfusion, fucoidin treated; NIC = nonischemic control.

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Fig 5. Comparison of coronary vascular resistance (mm Hg · mL⁻¹ · min⁻¹ · g⁻¹) in nonischemic control (NIC), ischemia–reperfusion (I-R), and ischemia–reperfusion, fucoidin treated (I-R FCN) groups. *p ≤ 0.05 compared with nonischemic control group.
Leukocyte Accumulation in Venules

During reperfusion, leukocyte accumulation in venules is mediated by leukocyte–endothelial cell adhesion molecule interaction [22]. The selectin family of adhesion molecules mediates the initial rolling and tethering attachment of leukocytes to the vascular endothelium. Weyrich and colleagues [23] examined the time course of adhesion molecule expression in cat myocardium after ischemia–reperfusion. They found that the expression of endothelial P-selectin was rapid, reaching maximum after 20 minutes of reperfusion. Thus, P-selectin is a good candidate for mediating the early component of reperfusion injury.

P-selectin (GMP-140, CD62P, PADGEM) is synthesized and stored in Weibel-Palade bodies of endothelial cells and is rapidly translocated to the cell surface in response to inflammatory mediators such as oxidants, thrombin, histamine, and platelet-activating factor [24]. The rapid mobilization of P-selectin to the endothelial surface mediates leukocyte tethering on endothelium in vivo [25]. L-selectin (LAM-1, CD62L, MEL-14) is constitutively expressed on circulating leukocytes and also plays a role in the initial phase of leukocyte attachment to postcapillary venules [17, 26]. The effects of monoclonal antibodies directed toward P- and L-selectin were examined in feline myocardium by Weyrich and associates [9] and Ma and colleagues [10]. They found that P- and L-selectin inhibition were effective in reducing myocardial necrosis, assessed after 90 minutes of ischemia and 270 minutes of reperfusion. In addition, Lefer and colleagues [8] found that treatment with a carbohydrate analog of the Sialyl Lewisα ligand, CY-1503, significantly reduced leukocyte accumulation in the myocardium of dogs subjected to 1.5 hours of ischemia and 4.5 hours of reperfusion.

These studies indicate that P- and L-selectin molecules are expressed during early reperfusion, and that selectin inhibition is cardioprotective when assessed several hours after reperfusion. However, these studies did not examine the effects of selectin inhibition on initial leukocyte accumulation in coronary capillaries and venules during the first minutes of reperfusion. Toward that aim, in the present study intravital microscopy was used to observe directly the effects of selectin inhibition on leukocyte accumulation in the postischemic microvessels during the first moments of reperfusion.

Fucoidin, derived from the marine algae Fucus vesiculosus, is a sulfated, fucosylated polysaccharide with a structure that is similar to the natural carbohydrate sequences recognized by the lectin domains of the selectins Sialyl Lewisα and Sialyl Lewisα. Because of this structural similarity, FCN binds nonselectively to P- and L-selectins and competitively inhibits binding [27]. Microvascular studies in noncoronary microcirculations have reported efficacy of FCN to reduce leukocyte accumulation after I-R. Using intravital microscopy, Kubes and associates [13] observed that FCN (25 mg/kg) significantly reduced leukocyte rolling and adhesion to cat mesentery venules after 60 minutes of low flow ischemia and 60 minutes of reperfusion. On the basis of this interesting work in the mesentery, we assessed leukocyte adhesion in the microcirculation of the heart using a similar concentration of FCN (0.36 mg/mL blood). We treated both the heart and the blood with FCN in an attempt to inhibit P-selectin on the vascular endothelium as well as L-selectin on the leukocytes. Similar to the results of Kubes and colleagues [13] in the mesentery, we found that FCN treatment was effective in significantly decreasing leukocyte adhesion to rat coronary venules subjected to 30 minutes of ischemia and 35 minutes of reperfusion.

Kubes and associates [13] also reported that during reperfusion, FCN was not effective in blocking leukocyte adhesion in venules in which shear rates decreased to less than 70% of control shears (612 per second), that is, less than about 430 per second. To test the mechanism responsible for this observation, they treated another group of animals with FCN and an antibody to the leukocyte β2-integrin adhesion molecule CD18. The endothelial ligand for CD18 is the intercellular adhesion molecule protein. In these experiments they found that under low shear rate conditions, whereas FCN was not effective in preventing leukocyte adhesion, anti-β2-integrin therapy abolished leukocyte adhesion to venules. They concluded that despite the significant reduction in leukocyte rolling and adherence with FCN, remaining rolling cells were able to adhere by way of a CD18-dependent mechanism in venules with reduced shear rates. Perry and Granger [28] also demonstrated that low shear rates permit an integrin-dependent adhesive interaction between leukocytes and nonischemic microvascular endothelium. In contrast to the findings of Kubes and colleagues [13] and Perry and Granger [28], we found that selectin inhibition with FCN was effective in inhibiting leukocyte adhesion to venules when postischemic hearts were reperfused under conditions of low blood flow with shear rates of about 100 per second. The results of this and our earlier study [7] indicate that low blood flow and reduced shear enhance unstimulated leukocyte adhesion to coronary venules. There may be inherent differences in leukocyte–endothelial interactions between the mesentery and the heart. It is possible that in the heart, the selectin family of adhesion molecules, and not integrin–endothelial intercellular adhesion molecule interactions, mediate low flow leukocyte adhesion to postischemic venules.

P-selectin [9] and L-selectin [10] inhibition were effective in preventing leukocyte-mediated reperfusion injury after ischemia–reperfusion in cat hearts. Assessment of injury in these studies was performed several hours after reperfusion. Although coronary blood flow was not assessed, similar experiments by these groups of investigators indicate that coronary blood flow was significantly reduced to 10% to 50% of control values in the first minutes of reperfusion [19, 29]. These results, combined with the results from our study, suggest that during reperfusion of the ischemic myocardium, P- and L-selectin inhibition may be effective under conditions of reduced flow.

We also observed that, although there was a significant washout of leukocytes in the venules of all groups during reperfusion, FCN treatment further reduced the persis-
ence of the leukocyte adherence compared with the other groups. These data indicate that the nature of the initial leukocyte adhesion is transient, and that the transient attachment may be selectin mediated.

**Leukocyte Accumulation in the Capillaries**

During ischemia–reperfusion, the mechanisms responsible for leukocyte retention in the coronary capillaries are likely different than those in the venules. Leukocytes appear to plug or trap in capillaries, whereas they adhere to the walls of the much larger venules. Earlier we demonstrated that under conditions of full flow reperfusion, treatment with pentoxifylline significantly decreased leukocyte retention in postischemic capillaries [15]. We suspect that pentoxifylline improved the deformability of WBCs, increasing their ability to traverse capillaries. In a subsequent study, we found that when postischemic hearts were reperfused at 10% of full flow, leukocyte accumulation in capillaries increased fivefold compared with controls [7]. In the present study, hearts were reperfused at low flow and the hearts and blood reperfusate were pretreated with FCN. Leukocyte accumulation in the capillaries was significantly decreased compared with the untreated group. These results indicate that adhesion mechanisms contribute to leukocyte retention in coronary capillaries early in reperfusion. The fact that in the fucoidin group leukocyte retention in capillaries was still greater than found in nonischemic controls indicates that other factors, such as blood cell deformation [15] and microvascular compression [30], also contribute to early leukocyte accumulation in capillaries in the heart.

With respect to leukocyte persistence, we found that after 35 minutes of reperfusion, fewer leukocytes were retained in the coronary capillaries of hearts treated with FCN. That is, pretreatment with FCN also promoted an increased washout of previously trapped cells. As in the venules, these results indicate that the initial accumulation of WBCs is selectin mediated. To our knowledge, this is the first report of selectin-mediated leukocyte retention in capillaries of any microvascular bed. Additional studies are necessary to elucidate the relative importance of selectins in both capillary and venular leukocyte retention during early reperfusion after ischemia.

**Leukocyte Accumulation Early in Reperfusion and the Recovery of Cardiac Function**

The notion that a reduction in leukocyte accumulation in the coronary microcirculation early in reperfusion may benefit overall cardiac function is supported by several studies. First, early investigations suggested that leukocyte depletion improves microvascular function [3] and the recovery of pump function after ischemia [2]. Second, a recent study by Miura and colleagues [5] found that selectin blockade with FCN resulted in improved recovery of left ventricular function, coronary blood flow, and myocardial oxygen consumption during the first hour of reperfusion after cold ischemia. Third, Omata and colleagues [31] reported that FCN treatment reduced myocardial infarct size and leukocyte accumulation in myocardial tissue 6 hours after reperfusion. Our findings suggest that the improvement in ventricular function and the reduction in infarct size observed in the above studies may be related to the ability of FCN to attenuate the initial microvascular leukocyte accumulation.

We hypothesized that a reduction in leukocyte accumulation with the selectin blocker FCN would result in an improvement in cardiac function, measured as coronary vascular resistance. However, we found that coronary vascular resistance was not reduced with FCN treatment, although leukocyte accumulation in both capillaries and venules was decreased in this group. Several factors may have accounted for this observation. First, it is known that capillary no-reflow is related to increased coronary vascular resistance [3]. In our study, FCN did not completely inhibit leukocyte retention in capillaries. The leukocytes remaining in the capillaries in this group could contribute to the observed increased resistance. Alternately, the increase in vascular resistance in both postischemic groups may be the result of the conditions of reperfusion, that is, the low blood flow state. Any benefit realized by a reduction in leukocyte accumulation in the FCN-treated group may have been obscured by an extension of the ischemic insult induced by the continuous low flow state [32, 33].

Although studies indicate that leukocytes contribute to reperfusion injury, other factors such as the conditions of reperfusion, can also modify the extent of injury. For example, a recent study by Sato and colleagues [11] examined the hypothesis that controlling the hydrodynamics of reperfusion (gradual reperfusion) would reduce postischemic coronary artery endothelial dysfunction and inhibit neutrophil accumulation in the area at risk. In dogs, the coronary artery was ligated for 60 minutes and reperfusion was initiated at full flow or gradually returned over 30 minutes. In the latter group, coronary artery blood flow was approximately 10% to 30% of control flow for the first 15 minutes of reperfusion. They found that gradual reperfusion reduced infarct size and attenuated macrovascular endothelial dysfunction. Surprisingly, they found that neutrophil accumulation, assessed by the tissue myeloperoxidase technique, increased in the area at risk. Sato and colleagues [11] concluded that although varying the conditions of reperfusion by controlling hydrodynamics may improve cardiac function, the full benefit of gradual reperfusion may not be realized in the presence of significant leukocyte accumulation. Similar to the findings of Sato and colleagues [11], we found that leukocytes rapidly accumulate during low flow reperfusion. Our direct observations of the microcirculation showed that the accumulation occurs in both the capillaries and postcapillary venules. Furthermore, our studies indicate that the early leukocyte accumulation is mediated by selectins.

In summary, efforts to minimize myocardial leukocyte-mediated reperfusion injury are warranted, especially in light of the number of clinical interventions in which low flow reperfusion may occur. We found that selectin inhibition with FCN reduced leukocyte adhesion to postischemic coronary venules reperfused at low flow. In
addition, FCN inhibited leukocyte retention in coronary capillaries during reperfusion. We also found that the persistence of leukocyte sequestration in both venules and capillaries was decreased with selectin inhibition, suggesting that the transient nature of early leukocyte accumulation is selectin mediated. These studies suggest that selectins, perhaps P- and L-selectin, mediate early leukocyte adhesion to venules and to some extent in coronary capillaries. These findings also suggest that selectin therapies may be beneficial in reducing initial leukocyte accumulation and early reperfusion injury in the heart.

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