IT IS WELL RECOGNIZED that sustained ischemia can induce cell death and tissue necrosis, which are mainly caused by energy insufficiency. Many studies have shown that tissue injury in many organs occurs not only during the ischemic period but also in the reperfusion period (20). Therefore, both the mechanism of and therapeutic intervention for reperfusion injury after ischemia have received considerable attention. However, the mechanism of reperfusion injury is multifactorial, and therapeutic interventions are not well established.

Ischemia-reperfusion injury in skeletal muscle may occur clinically after a release of aortic clamping during vascular reconstruction, a release of tourniquets during orthopedic surgery, or extrication of a trauma victim who is compressed with a heavy weight for a prolonged period (crush syndrome). Several reports have indicated that the pathophysiology of the injury varies according to the duration and grade of the ischemia (26, 28). Yokota et al. (37) demonstrated that cellular membrane injury persists after 60-min reperfusion following 90-min partial ischemia in rat skeletal muscle despite restoration of high-energy phosphates and recovery from lactic acidosis, and they have suggested that leukocyte-generated oxygen free radicals play a major role in reperfusion injury in skeletal muscle, because they showed that leukocyte depletion and oxygen free radical scavengers attenuated reperfusion injury. However, the precise mechanism of leukocyte-induced reperfusion injury in skeletal muscle microcirculation has not been determined.

In addition, pentoxifylline [PTXF, 1-(5-oxohexyl)-3,7-dimethylxanthine], a xanthine-derived phosphodiesterase inhibitor that increases intracellular cAMP, has been shown to increase peripheral blood flow by improving erythrocyte deformability and suppressing platelet aggregation (29). Recent studies have indicated that PTXF improves ischemia-reperfusion injury in many organs by attenuating neutrophil adhesion (sequestration) to the endothelial cells, production of reactive oxygen species, and platelet activation (1, 18, 27). However, the effects of PTXF on reperfusion injury in skeletal muscle after partial ischemia are not clear.

This study was designed to evaluate, through measurement of the resting transmembrane potential difference ($E_m$), whether PTXF attenuates reperfusion injury in skeletal muscle after partial ischemia, and, if so, to clarify the mechanism of PTXF action on leukocytes in reperfusion injury from the standpoint of postcapillary microcirculation. We measured 1) the resting $E_m$ of hindlimb skeletal muscle in rats 2) hemodynamics, including hemoglobin oxygenation (HbO$_2$), adherent leukocyte count (L$_a$), and rolling leukocyte count (L$_r$) in postcapillary venules, and 3) contralateral hindlimb blood flow (BF). On the basis of our results, the effects of PTXF on ischemia-reperfusion injury in the skeletal muscle are discussed.

**MATERIALS AND METHODS**

**Animal Preparation**

Male specific pathogen-free Wistar rats (250–300 g) were obtained from a commercial vendor (Nihon SLC, Hamamatsu, Japan) and were allowed free access to food and water. All animal experiments were conducted in accordance with the Animal Care and Use Committee of Osaka University Medical School. Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg body wt), followed by intraperitoneal supplements (15 mg/kg) as required, and were placed on a warming blanket at 37°C. The animals were allowed to breathe room air spontaneously. The right carotid artery was cannulated with an intravenous cannula (0.63-mm
outer diameter, Portex) for measurements of arterial pressure (AP) and heart rate (HR) and for blood sampling. The right external jugular vein was cannulated for fluid and drug administration. The rats received continuous intravenous administration of saline at a rate of 3 ml·kg⁻¹·h⁻¹ throughout the experiment. A midline laparotomy was performed, and the infrarenal aorta was isolated. Muscle fibers of the hindlimb adductor muscle were exposed through a 5-mm diameter skin incision. Subcutaneous tissue and overlying muscle fascia were dissected, and the defect was covered with saline. Heparin (400 U/kg) was infused, and the aorta was occluded by vascular clip (Bear TK5-1, Kyowa Precision Instruments, Ichikawa, Japan). The laparotomy wound was then closed. Ninety minutes after aortic clamping, the clip was released. Rats were monitored for 60 min after declamping.

Experimental Protocol 1

To evaluate whether PTXF attenuates ischemia-reperfusion injury in skeletal muscle, we measured the resting $E_m$, tissue water content, and tissue lactate content. Rats were randomly divided into four groups. Group 1 served as the ischemia-reperfusion group (IR, $n = 6$) and underwent the experimental procedure. Groups 2 and 3 were administrated a solution of PTXF (Hoechst J pan, Tokyo, J pan) intravenously at 20 ($P_{20}$, $n = 6$) and 40 ($P_{40}$, $n = 6$) mg/kg on clamping, followed by continuous infusion at 0.1 and 0.2 mg·kg⁻¹·min⁻¹, respectively. Group 4 rats underwent the same procedure as IR rats with the exception that the aorta was not occluded (Sham, $n = 5$).

We measured AP, HR, and arterial blood gases with a blood gas analyzer (ABL510; Radiometer, Copenhagen, Denmark), whole blood cell count using a hematology analyzer (Sysmex K-1000; Toa Medical Electronics, Kobe, Japan), and hindlimb skeletal muscle $E_m$ at three time points, i.e., just before aortic clamping (Baseline), just before aortic declamping (Ischemia), and 60 min after declamping (Reperfusion). In the IR, $P_{20}$, and Sham groups, we also measured serum electrolyte concentration using an electrolyte analyzer (EML100; Radiometer) at each time point. After this, hindlimb skeletal muscle was removed for determination of tissue lactate and water content.

Experimental Protocol 2

To evaluate the effect of PTXF on the circulation of reperfused skeletal muscle after ischemia, we analyzed the hemodynamics and leukocyte behavior of the involved postcapillary venules and hindlimb BF. Rats were randomly divided into three groups: an ischemia-reperfusion group (IR, $n = 8$), a PTXF-treated group ($P_{20}$, $n = 8$), and a sham-operated group (Sham, $n = 5$). In each group, rats were prepared as in experiment 1. Using intravital microscopy, we observed postcapillary venules with diameters of 13.0 ± 0.6 μm (mean ± SE) in the hindlimb adductor muscle at Baseline, Ischemia, and Reperfusion. We measured $L_a$ and $L_t$ at Baseline and Reperfusion, and we also measured vessel diameter (Dia), flowing erythrocyte velocity ($V_rbc$), HbO₂, and wall shear rate (WSR) at three time points. We observed two vessels in each rat, and the averages of the above-mentioned parameters were recorded. To confirm the microscopic data, BF in the contralateral hindlimb muscle was also measured at three time points using a laser-Doppler flowmeter with an LS-type fiber probe (FLO-C1; Neuroscience, Tokyo) by which the BF volume is given as the product of the measured density and the flow velocity of red blood cells in the tissue (19). The $D_{io}$, $V_{rbc}$, and BF measurements are expressed as the percentages of those at Baseline in each group.

Measurement of $E_m$

We measured resting $E_m$ using a modified Ling-Gerard microelectrode method previously described (14). Microelectrodes were made of borosilicate glass tubing (A-M Systems, Everett, WA) using a vertical tip puller (PN-3; Narishige Scientific Instrument, Tokyo). The diameter of the microelectrode was <1 μm, and its tip resistances were between 3 and 10 MΩ. The microelectrode was back-filled with 3 M KCl and mounted in a plastic holder containing a Ag/AgCl half-cell with a chamber filled with the same solution. This electrode was connected to an electrometer (model FD223; World Precision Instruments, New Haven, CT), and the amputated rat tail stump was immersed in saline with the indifferent electrode consisting of a second Ag/AgCl half-cell. Resting transmembrane potentials and tip resistances were recorded on a strip chart (SS-250F; Sekonic, Tokyo). Successful impalement was characterized by rapid upward deflection of the pen, indicating relative intracellular electronegativity, which was stabilized within 10 s and remained stable for 5 s. Individual muscle cells were impaled with the electrode using a micromanipulator (Narishige Scientific Instrument). About 15–20 cells were impaled at 2–4 points during each measurement.

Measurement of Tissue Water and Lactate Content

We took ~7 mm of adductor muscle from the hindlimb at 60 min after declamping. The muscle was weighed immediately as wet weight and then soaked in liquid nitrogen and freeze-dried for 48 h with a freeze-dry system (Labconco, Kansas City, MO) before it was weighed as dry weight. The tissue water content was expressed as:

$$\text{water content (\%)} = \left[\frac{\text{wt wt} - \text{dry wt}}{\text{wt wt}}\right] \times 100$$

The tissue lactate content was determined as follows. The freeze-dried muscle was ground to ~1 mm² in volume with a mortar made of onyx. The ground muscle was weighed and put into a test tube; 200–300 μl of 3% HClO₄ solution were added into the tube. The muscle was homogenized with a homogenizer (Polytron model K; Kinematica, Littau/Lucerne, Switzerland) and further with an ultrasonic homogenizer (model XL-2005; Heat Systems, Farmingdale, NY). Centrifugation of the solution was done at 50,000 rpm for 1 h at 4°C. Lactate concentration of the supernatant fluid was measured enzymatically using a Determiner LA kit (Kyowa Medics, Tokyo) with a spectrophotometer (model CL-770; Shimazu, Kyoto, J pan) to determine the tissue lactate content, reported as micromoles per gram of dry tissue weight.

Apparatus for Microscopic Observation

The muscle surface was epi-illuminated with a 150-W halogen lamp (fiber optic light source; Nikon Optical, Tokyo). The microcirculation was observed under a binocular microscope (STXM-S; Olympus, Tokyo) equipped with long-working-distance objective lenses (MPlan ×10 and ULWDMSPan ×50), in which the numerical aperture/working distances (in mm) were 0.30/9.00 and 0.55/8.10, respectively. The final magnification on a television monitor was ×450 with MPlan ×10, or ×2,250 with ULWDMSPan ×50. The diameter and length of the microvessels were measured on-line using an image processor (Nexus Qube 310; Nexus, Tokyo).
Microscopic Reflectance Spectrophotometry for Measurement of HbO2

The reflection spectrum from a spot 8 µm in diameter on the visual field of the microscope was recorded using the objective lens ULWDMSPPlan ×50 as follows. Reflected light from the spot was guided to a scanning/grafting spectrophotometer (USP-410; Unisoku, Osaka, Japan), through a thin quartz light guide (0.4 mm in diameter) inserted through a tiny hole in a cylindrical block attached to the microscope instead of the eye piece. The grating could be scanned by 500 steps in a desirable wavelength range (i.e., 500–600 nm), and the detector could count 1 · min -1 · µm of vessel length. We counted the number of rolling leukocytes (Lr), cells moving slower than erythrocytes in the vessel. Lr was expressed as the number of rolling leukocytes that passed by a given point for 1 min divided by Vmean. Both determinations were repeated three times at each time point.

RESULTS

Table 1. Mean arterial pressure and systemic leukocyte count

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>Leukocytes, per mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Ischemia</td>
</tr>
<tr>
<td>Sham</td>
<td>117 ± 8</td>
<td>114 ± 8</td>
</tr>
<tr>
<td>IR</td>
<td>116 ± 3</td>
<td>129 ± 4</td>
</tr>
<tr>
<td>P20</td>
<td>107 ± 6</td>
<td>120 ± 6</td>
</tr>
<tr>
<td>P40</td>
<td>101 ± 8</td>
<td>118 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. MAP, mean arterial pressure; leukocytes, systemic leukocyte count. Sham-operated group (Sham) had no aortic occlusion and no pentoxifylline (PTXF). Ischemia-reperfusion group (IR) had an aortic occlusion and no PTXF. P20 and P40 groups received bolus injection of PTXF (20 and 40 mg/kg, respectively) on aortic clamping followed by infusion at 0.1 and 0.2 mg·kg⁻¹·min⁻¹, respectively. No significant differences were noted between groups and stages.

Measurement of Flowing Erythrocyte Velocity and Adherent Leukocytes

Double-spot cross-correlation methods were used for estimating Vmean (36). A computer-controlled dual-channel red blood cell velocity measurement system (USP-500S; Unisoku, Tokyo) was used. The lights from two spots (8 µm in diameter) on a microvessel were guided through two separate quartz light guides (400 µm in diameter) to two photomultipliers connected to tiny holes in another cylindrical block attached to the microscope. The determination of Vmean was repeated three times during spectral recording, and the average velocity was calculated. WSR was calculated based on the Newtonian definition WSR = 8 × (Vmean/Dia), where Vmean = Vmean/1.6 (12).

The measurements of Lα and Lr were made from video recordings as follows. An adherent leukocyte was defined as one that remained stationary on the vessel wall for a minimum of 30 s (15). Lα was expressed as the number of leukocytes per 100 µm of vessel length. We counted the number of rolling leukocytes (Lr), cells moving slower than erythrocytes in the vessel. Lr was expressed as the number of rolling leukocytes that passed by a given point for 1 min divided by Vmean. Both determinations were repeated three times at each time point.

Statistical Analysis

Data are reported as means ± SE unless otherwise indicated. The statistical analysis consisted of analysis of variance followed by Student-Newman-Keuls test. Differences were considered significant when the P value was <0.05.

Table 2. Arterial blood gas analysis at 60 min after declamping

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 5)</th>
<th>IR (n = 6)</th>
<th>P20 (n = 6)</th>
<th>P40 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/dl</td>
<td>14.6 ± 0.5</td>
<td>13.3 ± 0.5</td>
<td>14.2 ± 0.2</td>
<td>13.6 ± 0.5</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>106.1 ± 14.9</td>
<td>110.7 ± 9.7</td>
<td>108.6 ± 4.9</td>
<td>110.4 ± 5.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.363 ± 0.021</td>
<td>7.359 ± 0.035</td>
<td>7.411 ± 0.028</td>
<td>7.379 ± 0.011</td>
</tr>
<tr>
<td>BE, meq/l</td>
<td>-2.7 ± 1.0</td>
<td>-3.4 ± 0.9</td>
<td>-4.7 ± 1.2</td>
<td>-5.6 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Hb, hemoglobin; BE, base excess. No significant differences were noted between groups.
Effects of pentoxifylline on reperfusion injury

Hindlimb BF. Figure 4 shows changes in BF in the contralateral hindlimb muscle. During the ischemia period, BF was significantly reduced to ~22% of Baseline in both the P20 and the IR group, and no significant difference was noted between the two groups. At Reperfusion, BF in the IR group recovered to 83% of Baseline, but this was significantly lower than at Baseline. BF in the P20 group during the reperfusion period recovered to its baseline level and was significantly higher than in the IR group.

Lₐ and Lₐ. Figure 5 shows changes in Lₐ and Lₐ. At Baseline, no significant differences were noted in Lₐ among the three groups. At Reperfusion, Lₐ in the IR group increased significantly, but Lₐ in the P20 group was significantly lower than in the IR group. There was no significant difference in Lₐ among the P20 and Sham groups at Reperfusion (Fig. 5A). There were no significant differences in Lₐ among the three groups at Baseline. At Reperfusion, Lₐ in the IR group was significantly higher than in the Sham group. Lₐ in the P20 group had a tendency to be lower than in the IR group, although the difference was not significant. No significant difference was noted in Lₐ between the P20 and the Sham group at Reperfusion (Fig. 5B).

DISCUSSION

In experiment 1, we demonstrated that during the reperfusion period 1) the resting Eₐ continued to be depolarized in the IR group, whereas it was improved in the PTXF-treated groups; 2) the water content in the IR group increased, whereas in the PTXF-treated groups it did not change; and 3) there was no significant difference in tissue lactate content between the IR and PTXF-treated groups. In experiment 2, we demonstrated that 1) Lₐ increased after ischemia-reperfusion; PTXF attenuated this increase, and Lₐ in the PTXF group had a tendency to be lower than in the IR group; 2) both V_rbc and WSR decreased after ischemia-reperfusion, and PTXF attenuated this decrease, al-

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Fig. 1. Changes in resting transmembrane potential difference (Eₐ) of hindlimb muscle in the Sham, IR, P20, and P40 groups. P20 and P40 groups received 20 and 40 mg/kg bolus injection of pentoxifylline (PTXF), respectively, on aortic clamping followed by infusion at 0.1 and 0.2 mg·kg⁻¹·min⁻¹, respectively. Ischemia-reperfusion (IR) group had an aortic occlusion and no PTXF. Sham-operated group had no aortic occlusion and no PTXF. Values are means ± SE; n, no. of rats. *P < 0.01 vs. IR; § P < 0.05 vs. Baseline; † P < 0.01 vs. Sham. Eₐ in IR, P20, and P40 groups was depolarized significantly (P < 0.01 vs. Baseline) at 90 min after clamping. During reperfusion period, Eₐ in IR group continued to be depolarized, whereas Eₐ in P20 and P40 groups was significantly repolarized (P < 0.05), but not to baseline levels.

Figure 2B shows the tissue water content of hindlimb muscle at 60 min after aortic declamping. Water content in the IR group was significantly higher than in the Sham group; water content in the P20 group was not significantly different from that in the Sham group.

Experiment 2

Effects of aortic clamping-declamping and PTXF on skeletal muscle microcirculation. Figure 3 shows changes in Dia, HbO₂, V_rbc, and WSR. Dia in the P20 group increased significantly during the reperfusion period in comparison to Dia at Baseline and Ischemia. However, no significant differences in Dia were noted among the three groups during reperfusion (Fig. 3A). HbO₂ decreased significantly during the ischemia period in both the IR and the P20 group, but returned to Baseline levels at Reperfusion (Fig. 3B). V_rbc decreased significantly in the IR and P20 groups at Ischemia, and no significant difference was noted between the two groups. At Reperfusion, V_rbc in the IR group was significantly lower than at Baseline, and V_rbc in the P20 group was significantly higher than at Baseline and in the IR group (Fig. 3C). During the reperfusion period, WSR in the IR group was significantly lower than at Baseline, and WSR in the P20 group was significantly higher than in the IR group (Fig. 3D).
though there were no significant differences in Dia and HbO₂; and 3) BF in the IR group did not recover to its baseline level at Reperfusion, whereas in the P20 group it recovered fully.

The grade of ischemia-reperfusion injury in skeletal muscle has been evaluated using various techniques, e.g., morphological (33), functional (muscle contractile) (22), and histochemical (10) techniques. We used an electrophysiological approach by measuring the value of resting $E_m$ with respect to the grade of ischemia-reperfusion injury. The resting $E_m$ of skeletal muscle is known as a sensitive and useful indicator of cell membrane injury (14). Oredsson et al. (26) showed energy charge and tissue lactate content to be strongly correlated with resting $E_m$ during ischemia (1-, 2-, 4-, and 6-h ischemia) in an isolated rabbit hindlimb perfusion model, and they demonstrated that resting $E_m$ was not repolarized significantly, although energy charge and tissue lactate content were significantly restored during reperfusion after 2- and 4-h ischemia. Our results are in agreement with theirs (Figs. 1 and 2A). The values of the resting $E_m$ at Baseline in our study were similar to those values in the control state reported by Oredsson et al. (26), Yokota et al. (37), and Yoshioka et al. (38). Thus we consider reperfusion...
injury to be caused by factors other than energy insufficiency and/or intracellular acidosis. In the present study, resting $E_m$ in the P20 and P40 groups was significantly repolarized (−85.6 ± 1.5 mV and −86.2 ± 2.8 mV, respectively) during the reperfusion period (Fig. 1), indicating an improvement in the reperfusion-associated cellular membrane injury. There was no significant difference between the P20 and P40 groups in resting $E_m$ during reperfusion. The values of resting $E_m$ during the reperfusion period in the PTXF-treated groups were similar to those in the groups reported by Yokota et al. (37) and to those in a hypothermia group reported by Yoshioka et al. (38). These reports suggest that sufficient PTXF concentration was made available by the manner of administration in the P20 group in our ischemia-reperfusion model.

Leukocytes have been shown to play a major part in reperfusion injury. Therefore, interventions against leukocyte action have received considerable attention. For instance, pretreatment with superoxide dismutase and catalase showed an improvement in the resting $E_m$ in reperfused rat skeletal muscle (37). Korthuis et al. (21) reported that reperfusion with leukocyte-depleted blood prevented an increase in vascular permeability and resistance in canine gracilis muscle. Monoclonal antibody CD11b/CD18 prevented an increase in vascular permeability and resistance in canine gracilis muscle (7) and neutrophil accumulation in reperfused rat muscle.

PTXF is one of the phosphodiesterase inhibitors that have been reported to increase intracellular cAMP and reduce superoxide anion production by both monocytes and polymorphonuclear cells dose dependently in vitro (5). Endres et al. (13) indicated that PTXF led to a marked increase in cAMP levels, whereas cGMP levels were only marginally elevated in lipopolysaccharide-stimulated human monocytes. PTXF has received considerable attention with respect to its action on leukocytes in many organs (9, 11, 31). Reignier et al. (27) reported that after reperfusion, myeloperoxidase activity and blood neutrophil count were lower with PTXF than with saline, and changes in the filtration coefficient were correlated to the percent changes in blood neutrophils during reperfusion. They suggested that this effect may be mainly caused by a decrease in sequestration of neutrophils in the lung during reperfusion. Their group also reported that PTXF prevented endothelial injury during ischemia-reperfusion by decreasing neutrophil sequestration in isolated perfused rat and rabbit lungs and in pigs after left lung allotransplantation (9).

Water content in the P20 group during reperfusion was significantly lower than in the IR group. This indicates that PTXF attenuated not only cellular membrane injury of myocytes but also tissue edema during the reperfusion period, although we could not prove directly where water retention occurred in the tissue, in interstitial space, intracellular space, or in both. These results were compatible with these of several other studies. Carter et al. (8) demonstrated that PTXF reduced alveolar injury and subsequent protein leakage and improved cardiac output when administered after 30 min of intestinal ischemia. Okabayashi et al. (25) also reported that PTXF in the canine allograft model attenuated the increase in water content and myeloperoxidase activity in the lung after donor lungs were flushed with modified Euro-Collins solution and stored in an inflated state for 18 h at 1°C. Nakagawa et al. (24) indicated that PTXF inhibited N-formylmethionyl-leucyl-phenylalanine-induced macromolecular leakage in rat cremaster muscle.

Using intravital microscopy, Forbes et al. (15) reported that the number of rolling and adherent leukocytes increased in skeletal muscle during the reperfusion period after ischemia. Hanazawa et al. (17) reported that treatment with PTXF prevented accumulation of rolling and sticking leukocytes after reperfusion in the venules of rat cremaster muscle. Our study showed that $L_a$ increased significantly in the IR group during reperfusion and that PTXF attenuated leukocyte adhesion to endothelial cells of postcapillary venules in the reperfused hindlimb. $L_r$ in the PTXF group also had a
tendency to be lower than in the IR group, although the difference was not significant (Fig. 5). In addition, total leukocyte counts in our experiment 1 did not differ between groups, suggesting that PTXF attenuated the number of activated leukocytes. Our results were compatible with those of Barroso-Aranda and Schmid-Schönbein (3), who reported that PTXF reduced the activated polymorphonuclear neutrophil count, although the total polymorphonuclear neutrophil count was not affected by PTXF in a hemorrhagic shock model.

It is naturally assumed that reperfusion-associated endothelial cell injury participates in leukocyte adhesion during the reperfusion period. Recently, Formigli et al. (16) reported the expression of E-selectin on the venular endothelium of reperfused skeletal muscle in patients who were undergoing reconstructive surgery for abdominal aortic aneurysm. Boldt et al. (6) demonstrated that PTXF suppressed plasma levels of soluble E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in multiple organ failure patients. In these adhesion molecules, the peak expressions of ICAM-1 and VCAM-1 occurred ~12 and 24 h, respectively, after cytokine stimulation, and E- and P-selectin have been shown to be expressed within a few hours (16). Ascer et al. (2) reported an increased level of interleukin 1 (IL-1) in the venous effluent in canine reperfused skeletal muscle. Furthermore, PTXF has been shown to reduce the production of tumor necrosis factor-α and IL-1 (32), which stimulate the expression of E-selectin on the venular endothelium. Thus we considered the possibility that some adhesion molecules (such as the selectin family) on the venular endothelium may also be involved in our acute-phase study, and PTXF may attenuate an increase in the expression of these adhesion molecules.

Leukocyte migration toward myocytes, as well as leukocyte activation and adhesion, would be essential for short-lived oxygen free radicals to induce cellular membrane injury in myocytes. Suval et al. (34) demonstrated, using an electron microscope, extravasation of leukocytes to the interstitial space after 1 h of reperfusion following 2 h of ischemia in rat skeletal muscle. In the present study, such migration of leukocytes was also expected to occur, although we did not measure it. LVE was significantly suppressed and resting Emax was significantly restored in the P20 group during the reperfusion period, so the migration of leukocytes might have been suppressed in the P20 group.

During the ischemia period, Ve and HbO2 in the observed vessels were significantly reduced in both groups, and no significant differences were seen between the IR and P20 groups. This indicates that PTXF does not affect perfusion of the postcapillary venules in skeletal muscle during ischemia. In our partial-ischemia model, BF was significantly reduced but not stopped during the ischemia period, and the no-reflow phenomenon as described by Schmid-Schönbein (30) was not observed during the ischemia-reperfusion period. During the reperfusion period, Ve and HbO2 were significantly restored in both groups, and Ve and WSR in the P20 group were significantly higher than in the IR group. However, venular HbO2 levels were not changed, and the increase in BF with PTXF was modest. In addition, it is likely that BF in the IR group was more than adequate to support normal oxidative metabolism. We considered, therefore, that the improvement in hindlimb BF alone could not account for the reduction in reperfusion injury, because the improvement had no significant influence on HbO2 in the postcapillary venules and the tissue lactate content in the Sham, IR, and P20 groups. Our data suggest that PTXF exerts its effect by some mechanism other than increase in BF.

Horton et al. (18) reported that PTXF prevented ischemia-reperfusion-mediated cardiac dysfunction in the same manner as free radical scavengers (superoxide dismutase and catalase). Adams et al. (1) demonstrated that the administration of PTXF before reperfusion of ischemic skeletal muscle significantly decreased the extent of muscle necrosis and platelet-activating factor levels in the venous effluent at all times, including reperfusion. Berens et al. (4) reported that the glomerular filtration rate was significantly greater in kidneys administered PTXF than in controls after 40 min of postocclusion reperfusion. Pretreatment of kidneys with indomethacin, a nonspecific cyclooxygenase inhibitor, blocked the protective effects of PTXF. Myers et al. (23) also reported that PTXF exerted a protective effect against severe mesenteric ischemia-reperfusion injury by maintaining release of splanchnic prostaglandin I2, a potent endogenous splanchnic vasodilator. These results suggest that PTXF improves ischemia-reperfusion injury by attenuating sequestration of neutrophils to the skeletal muscle and production of chemical mediators such as reactive oxygen species and platelet-activating factor, as well as by stimulating prostaglandin synthesis.

In summary, the present study clearly indicates that PTXF attenuated reperfusion-associated membrane injury and tissue edema and that PTXF suppressed leukocyte adhesion and improved hindlimb blood flow during the reperfusion period.

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