PR-39, a proline/arginine-rich antimicrobial peptide, prevents postischemic microvascular dysfunction

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Korthuis, Ronald J., Dean C. Gute, Frank Blecha, and Chris R. Ross. PR-39, a proline/arginine-rich antimicrobial peptide, prevents postischemic microvascular dysfunction. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1007–H1013, 1999.—We and others have previously demonstrated that intestinal ischemia-reperfusion (I/R) is associated with a large increase in oxidant production that contributes to microvascular barrier disruption in the small bowel. It has been suggested that the bulk of tissue damage during reperfusion can be attributed to adherent, activated neutrophils. From these observations, we hypothesized that pretreatment with PR-39, an endogenous neutrophil antibacterial peptide that is also a potent inhibitor of the neutrophil NADPH oxidase, would prevent postischemic oxidant production and the development of oxidant-dependent sequelae to I/R such as increased venular protein leakage. To test this postulate, oxidant production, venular protein leakage, leukocyte adhesion, and leukocyte emigration were monitored during reperfusion in control (no ischemia) rat mesenteric venules and in mesenteric venules subjected to I/R alone or PR-39 + I/R. Treatment with a single intravenous bolus injection of PR-39 (administered at a dose to achieve an initial blood concentration of 5 µM) abolished I/R-induced leukocyte adhesion and emigration in vivo. In vitro studies indicated that PR-39 prevents platelet-activating factor-induced neutrophil chemotaxis as well as phorbol myristate acetate (PMA)-stimulated intercellular adhesion molecule-1 expression by cultured endothelial cells. PR-39 pretreatment of rat neutrophils also blocked PMA-stimulated neutrophil adhesion to activated endothelial monolayers. In vivo, I/R was associated with a marked and progressive increase in oxidant production and venular protein leakage during reperfusion, effects that were abolished by PR-39 treatment. The results of this study indicate that PR-39 completely abolishes postischemic leukocyte adhesion and emigration. The time course for inhibition of oxidant production by PR-39 suggests that its antiadhesive properties account for this effect of the peptide. PR-39 may thus be therapeutically useful for prevention of neutrophil adhesion and activation during the postischemic inflammatory response.

leukocyte adhesion; leukocyte emigration; venular protein leakage; oxidant production; intravital microscopy; intercellular adhesion molecule-1

A LARGE BODY OF EVIDENCE indicates that reactive oxygen species contribute to the pathogenesis of ischemia-reperfusion (I/R) injury in a variety of organs, including the small intestine (7, 8, 14). On the basis of these studies, it has been proposed that xanthine oxidase-derived oxidants contribute to the formation of proinflammatory stimuli such as platelet-activating factor, leukotriene B4, and C5a, oxidative inactivation of anti-adhesive molecules such as nitric oxide, and the expression of endothelial cell adhesion molecules such as P-selectin and intercellular adhesion molecule-1 (ICAM-1). These events lead to the recruitment of leukocytes to the previously ischemic region during reperfusion. On activation, these inflammatory phagocytes produce tissue injury through the release of a much larger burst of reactive oxygen species via the action of NADPH oxidase as well as hydrolytic enzymes such as elastase (7, 8, 14).

PR-39 is a recently described proline-arginine (PR)-rich antimicrobial peptide that also is a potent down-regulator of the NADPH oxidase present in neutrophils (16, 17). In addition, PR-39 blocks high K+-induced reactive oxygen production by cultured endothelial cells and isolated, perfused rat lungs (2). Although PR-39 blunts oxidase activity in whole cell and in cell-free systems, its ability to ameliorate reperfusion injury has not been investigated. In the studies reported here, we found that PR-39 completely blocks postischemic oxidant production and venular protein leakage in rat mesenteries subjected to I/R. We also observed that PR-39 completely abrogated postischemic neutrophil adhesion and transvascular emigration, suggesting that this peptide modulates I/R injury via mechanisms in addition to its known effect on the neutrophil NADPH oxidase.

METHODS

Male Sprague-Dawley rats weighing between 150 and 250 g were anesthetized by intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body wt). After a surgical plane of anesthesia was attained in the rats, a tracheotomy was performed to maintain a patent airway during the experiment. The right carotid artery and the left jugular vein were cannulated for monitoring systemic arterial blood pressure and administering fluorescein isothiocyanate (FITC)-labeled albumin (Sigma) and supplemental doses of anesthetic, respectively. After a midline abdominal incision was made, the renal vascular pedicles were ligated to prevent the renal elimination of PR-39. This procedure was performed in all animals. A loop of mesentery was then exteriorized and prepared for microscopic observation as described previously (6, 13). Briefly, a jejunal loop of mesentery was draped over a 2-cm² optically clear viewing pedestal. The exposed bowel wall was covered by bicarbonate buffer-saturated gauze, and the mesentery was superfused with bicarbonate-buffered saline (37°C, pH 7.4) that was bubbled with a mixture of 95% N2-5% CO2. Body temperature was maintained with a thermostatically con-
trolled heat lamp. The preparation was then transferred to the microscope stage for videomicroscopic observation.

Intravital microscopy. The mesenteric microcirculation was viewed using an inverted microscope with a ×40 immersion objective lens (Nikon Optiphot, Japan). The mesentery was transilluminated with a 12-V, 100-W direct current-stabilized light source. A charge-coupled device camera (Hamamatsu Photonics, C2000–60, Japan) mounted on the microscope projected the image onto a television monitor (Sony, PVM-2030, Japan), and the images were recorded using a video cassette recorder (Mitsubishi, HS-U65, Japan). A video time-date generator (Panasonic, WJ-810, Japan) projected the time, date, and stopwatch function onto the monitor.

Single unbranched venules with diameters ranging between 20 and 30 µm and lengths >150 µm were selected for study. Venular diameter (Dv) was measured either on- or off-line using a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX). Red blood cell centerline velocity (VRC) was measured by using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University). The velocimeter was calibrated against a rotating glass disk coated with rat erythrocytes. Venular wall shear rate (WSR) was calculated from the newtonian definition: 

\[ WSR = \frac{8}{\pi D_v} \frac{V_{\text{mean}}}{D_v} \]

where \( V_{\text{mean}} \) is the centerline velocity/1.6 (1, 6, 13).

The number of adherent and emigrated leukocytes was determined off-line during playback of videotaped images. A leukocyte was considered to be adherent to venular endothelium if it remained stationary for a period ≥30 s (1, 6, 13). Adherent leukocytes were expressed as the number per 100-µm length of venule. Leukocyte emigration was determined by counting the number of extravasated leukocytes per microscopic field (per 150-µm venular length) based on video images of the extravascular compartment immediately adjacent to the venule under study. The number of rolling leukocytes was quantified by counting the number of leukocytes that rolled past a fixed point on the television monitor per 10-min observation period.

To quantify albumin leakage across mesenteric venules, 50 mg/kg of FITC-labeled bovine serum albumin (Sigma) were administered intravenously to the animals 30 min before each experiment (6, 13). Fluorescence intensity (excitation wavelength, 420–490 nm; emission wavelength, 520 nm) was detected by using a silicon-intensified target camera (C-2400–08, Hamamatsu Photonics). The fluorescence intensity of FITC-albumin within the venule under study (Iv) and in adjacent perivenular interstitium (Ii) was measured at various times after the administration of FITC-albumin with a computer-assisted digital imaging processor (NIH Image 1.35 on a Macintosh computer). The window used to measure average fluorescence intensities within and adjacent to the venule was set at 50-µm length and 20-µm width. An index of venular albumin leakage was determined from the I1-to-Ii, ratio at specific intervals during the experiment; i.e., baseline and 30 and 60 min of prolonged reperfusion or equivalent nonischemic times.

The oxidant-sensitive fluorescent probe dihydrorhodamine (DHR) (Molecular Probes) was added to the mesenteric superfuse (10 µM) in some experiments to monitor oxidant stress (15). The fluorochrome was visualized by using the same microscope and image analysis system described above. DHR fluorescence intensity was monitored in a region of mesentery that was equivalent to twice the area of the venule under observation. An image processor was used to monitor fluorescence intensity just before (baseline value, Ib) and after treatment (Ii). The ratio of Ii to Ib was used as an index of oxidative stress in mesenteric tissue (15).

Experimental protocols. After we completed the surgical preparation and placed the rat on the microscope stage, the mesentery was allowed to stabilize for 15 min. The mesentery was superfused (1.25 ml/min) with bicarbonate-buffered saline throughout the protocol. In separate groups of experiments, either FITC-albumin was administered intravenously or DHR superfusion was begun 15 min before basal video recordings and measurement of all parameters (arterial blood pressure, \( V_{\text{RC}} \), and \( D_v \)). Images from the mesenteric preparation were then recorded on videotape for 10 min for subsequent analysis. Once basal measurements were obtained, the mesentery was subjected to 20 min of ischemia followed by 1 h of reperfusion (I/R). Ischemia was produced by means of a snare placed around the superior mesenteric artery 5–7 arcades proximal to the vessel of interest. This produced a small area of regional ischemia, which prevented the systemic complications induced by subjecting large portions of the bowel to ischemia and reperfusion (6). All of the aforementioned variables were again recorded between minutes 20–30 and minutes 50–60 of the prolonged reperfusion period. All variables were measured at comparable time points in control animals (no ischemia) and in rats subjected to I/R only. For animals treated with PR-39, the peptide was dissolved in saline (0.5 ml) and administered intravenously just after ligation of the renal vessels to produce a blood concentration of 5 µM, calculated assuming blood volume represents 6% of body weight. After obtaining baseline measurements, we induced regional mesenteric ischemia, and all variables were measured at the time points described above.

Preparation of rat neutrophils. Whole blood, anticoagulated with acid citrate dextrose, was collected via cardiac puncture from male, halothane-anesthetized Sprague-Dawley rats weighing 200–350 g. Neutrophils were prepared using dextran sedimentation, density gradient centrifugation in Ficoll-Hypaque, and hypotonic lysis for removal of contaminating red blood cells, as described by Clark and Nauseef (5). Neutrophils from each animal were purified and studied separately for all in vitro studies. Each animal routinely yielded 6–8 × 10⁶ total neutrophils, with a purity of 95–98% and viability of 90%. Purity and viability were assessed using a hemocytometer and trypan blue staining.

Neutrophil chemotaxis assay. Purified rat neutrophils were prepared and suspended in RPMI 1640 medium at a final concentration of 5 × 10⁶ cells/ml. Chemotactic activity of PR-39 was determined using a microchemotaxis assay as previously described (9), except that neutrophils were exposed to chemotactic agent for 15 min.

Superoxide anion production assay. Whole cell O₂⁻ production by peripheral rat neutrophils was determined using a proprietary enhanced luminol reagent (Diogenes; National Diagnostics). Neutrophils (1 × 10⁶ cells/ml; 1 ml) suspended in Hanks' buffered saline solution containing 1 mM Ca²⁺, were placed in polycarbonate tubes, and 200 µl of reconstituted Diogenes reagent was added. PMA was then added to a final concentration of 50 ng/ml, and luminescence was measured over 30 min in a liquid scintillation counter operating in single photon-counting mode. For each animal's neutrophil preparation, control (no PR-39) aliquots were compared with aliquots pretreated with 5 µM PR-39 for 1 h at 37°C. Total oxidative burst activity over the 30-min observation period was determined by graphically calculating integrated light output values using the PC version of NIH Image (Scion Image; Scion). Because total superoxide production varies considerably between animals, individual data points for each paired experiment were normalized to the peak superoxide production of that experiment before integration.
The normalized superoxide production for PR-39-treated rats was compared with a hypothetical mean of 100%, using a two-sided t-test (GraphPad Instat v. 3.00; GraphPad Software). Statistical significance was defined at \( P \leq 0.05 \).

ICAM-1 expression in vitro. To determine the ability of PR-39 to block endothelial cell adhesion molecule expression, we used an in situ enzyme-linked immunosassay for cell surface ICAM-1 (19). Human umbilical vein endothelial cells (HUVEC) were grown to confluence on glass coverslips in 24-well plates, treated with 5 \( \mu \)M PR-39 for 2 h, and then stimulated with phorbol myristate acetate (PMA) (1 \( \mu \)g/ml) for 4 h. After the treatment, cell layers were rinsed with phosphate-buffered saline (PBS) and then fixed with 1% paraformaldehyde in PBS for 15 min at room temperature. The fixed cells were then rinsed three times with PBS and then incubated for 30 min at 37°C in primary antibody (mouse anti-human CD54 ICAM-1, Southern Biotechnical) diluted in PBS-0.1% nonfat dry milk. Cells were rewarshed three times in PBS and then incubated for 30 min at 37°C in secondary antibody (goat anti-mouse-peroxidase conjugate; Boehringer) diluted 1:1,000 in PBS/nonfat dry milk. After three final washes with PBS, coverslips were placed in fresh 24-well plates and substrate (o-phenylenediamine dihydrochloride in citrate-phosphate buffer, pH 5.0) was added to each well for 10 min at room temperature. Plates were read at 492 nm using an automated plate reader, and optical density readings were corrected for absorbance of secondary antibody only.

In vitro leukocyte-endothelial cell adhesion assay. A static adhesion assay was used to test the ability of PR-39 to block the adhesion of human neutrophils to HUVEC monolayers (18). Cells were grown to confluence on gelatin-coated coverslips and pretreated with interleukin-1 (10 U/ml; 4 h). A suspension of \( ^{51} \)Cr-labeled human neutrophils, pretreated with 5 \( \mu \)M PR-39 or vehicle for 2 h and then stimulated with 0.1 \( \mu \)g/ml PMA for 20 min, was injected into the chamber and allowed to settle onto the endothelial monolayer. After 30 min, the cells were treated with 0.5 ml of NaOH (0.1 M), and this solution was placed in scintillation tubes. \( ^{51} \)Cr activity was counted using a gamma counter. Data are expressed as counts per minute and represent the mean of six coverslips.

Statistical analysis. All values obtained in this study are expressed as means \( \pm SE \). With the exception of the study measuring superoxide anion production by rat neutrophils, the data were initially analyzed using a one-way analysis of variance. To identify which groups were statistically different, Bonferroni’s post hoc test was employed. Statistical significance was defined at \( P < 0.05 \).

RESULTS

Effect of PR-39 treatment on neutrophil-endothelial cell interactions. Figure 1 illustrates the changes in the number of adherent (Fig. 1A) and emigrated (Fig. 1B) leukocytes observed in mesentery subjected to I/R alone or PR-39 + I/R relative to values obtained at comparable time points in the time-control (no ischemia) experiments. I/R elicited a marked and progressive increase in the number of adherent and emigrated leukocytes compared with the values obtained at comparable time points in the time-control experiments. The I/R-induced increases in leukocyte adherence and emigration were abolished by treatment with PR-39. Although not statistically significant, I/R was also associated with a threefold increase in the number of rolling leukocytes (242.4 \( \pm \) 71.4 leukocytes/10 min) relative to that noted in nonischemic preparations (72.3 \( \pm \) 24.7 leukocytes/10 min). This effect was also abolished by PR-39 treatment (31.4 \( \pm \) 16.9 leukocytes/10 min).

The effects of PR-39 on PMA-induced ICAM-1 expression and neutrophil adhesion to cultured endothelial cells are presented in Fig. 2A and B. ICAM-1 expression was increased twofold in endothelial cell monolayers exposed to PMA for 4 h compared with that of naive cells (Fig. 2A). PMA also induced an increase in neutrophil adhesion to endothelial cell monolayers. The PMA-induced increases in endothelial ICAM-1 expression and neutrophil adhesion to endothelial monolayers were abolished by PR-39 treatment (Fig. 2A and B, respectively). Cytotoxicity assays using HUVEC cells exposed to 0.01–100 \( \mu \)M PR-39 for 1 h revealed that peptide treatment had no effect on cell viability (data not shown).

We have reported that PR-39, used alone, has chemotactic activity for pig neutrophils (9), a property that may have relevance in the recruitment of neutrophils by activated endothelium during the I/R cascade. Figure 3 illustrates the chemotactic response of rat neutro-
To PAF, PR-39, and the combination of PAF and PR-39. Both stimuli elicited a chemotactic response when used alone. When rat neutrophils were exposed to PAF and PR-39 simultaneously, the chemotactic index was decreased 41% and 18% compared with PAF and PR-39, respectively. These results suggest that PR-39 may act as an inhibitor of neutrophil chemotaxis when present in the complex environment of the I/R response.

Effect of PR-39 on oxidant production and microvascular barrier integrity. I/R was associated with an increase in oxidant production of 2.7- and 4.3-fold relative to baseline (before ischemia) at minutes 30 and 60 of reperfusion (Fig. 4A). Consistent with its ability to inhibit superoxide release by isolated pig neutrophils, PR-39 pretreatment abolished the I/R-induced increases in mesenteric oxidant production. We also monitored DHR oxidation during the first 10 min of reperfusion but noted no increase in the production of reactive oxygen species at this time. To confirm rat neutrophils as a potential target for oxidase inhibition,

Fig. 2. PR-39 prevents phorbol myristate acetate (PMA)-induced intercellular adhesion molecule-1 (ICAM-1) expression by (A) and neutrophil adhesion to (B) human umbilical vein endothelial cell monolayers. *Values statistically different from those obtained in cells not exposed to PMA (Control) at \( P < 0.05 \); + values statistically different from those obtained in cells exposed to PR-39 alone; # values statistically different from those obtained in cells exposed to PMA alone.

Fig. 3. PR-39 blunts neutrophil chemotactic response to platelet-activating factor (PAF). Simultaneous treatment of neutrophils with PAF (100 ng/ml) and PR-39 (10 \( \mu M \)) reduced chemotactic response 41% compared with PAF alone. *Values statistically different from PAF alone at \( P < 0.05 \).

Fig. 4. A: PR-39 blocks oxidant production of dihydrorhodamine (DHR oxidation) in mesenteries subjected to I/R. Control, data obtained at equivalent time points in time-matched controls. *Values statistically different from corresponding values in mesenteries subject to I/R alone at \( P < 0.05 \). B: PR-39 also attenuates superoxide production by isolated rat neutrophils stimulated with PMA (100 ng/ml). Control, superoxide production by non-PR-39-treated cells.
we tested the effect of PR-39 treatment on isolated cells stimulated with PMA. Pretreatment for 1 h with 5 µM PR-39 produced an 87% reduction in PMA-induced superoxide production. A representative comparison in one animal is shown in Fig. 4B. Concurrent treatment of neutrophils with PMA and superoxide dismutase reduced the luminescence signal by 98.8% (data not shown), indicating the specificity of the Diogenes reagent for the superoxide anion.

Figure 5 summarizes the changes in venular protein leakage (VPL) in control, I/R, and I/R + PR-39 groups. I/R resulted in an increase in mesenteric VPL (28.6 ± 1.8% at minute 30 of reperfusion and 47.3 ± 7.1% at minute 60 of reperfusion) relative to that seen at equivalent time points in control (nonischemic) preparations (9.7 ± 0.9% and 15.3 ± 5.5%, respectively). The I/R-induced increase in VPL was blocked by pretreatment with PR-39 (14.7 ± 1.9% and 22.4 ± 5.5%, respectively).

Mean values for leukocyte adhesion and emigration, venular protein leakage, and oxidant production at baseline conditions in each of the groups were not different. Results of the time-control (no ischemia) experiments indicated that none of these variables were altered during the experimental protocol.

Average values for venular diameter did not change during the protocol in any of the experimental groups. I/R induced reductions in both erythrocyte velocity and venular wall shear rate, effects that were not improved by PR-39 (data not shown). The lack of effect of PR-39 on erythrocyte velocity and venular wall shear rate indicates that the antiadhesive effect of this peptide was not due to altered hydrodynamic forces.

DISCUSSION

A large body of evidence indicates that leukocytes play a major role in the development of cellular dysfunction and necrosis in tissues exposed to I/R (6–8, 14). For example, rendering animals neutropenic before I/R largely prevents postsischemic tissue injury. Prevention of leukocyte rolling with antibodies directed against P-selectin is also quite effective in reducing reperfusion injury (7, 8, 14). In addition, blockade of leukocyte-endothelial cell adhesive interactions by administration of monoclonal antibodies that bind to functional epitopes on the leukocyte integrin CD11/CD18 or its counter ligand on endothelial cells ICAM-1 prevents I/R-induced firm (stationary) leukocyte adhesion and attenuates 1) postsischemic microvascular barrier disruption in the small intestine, lung, and skeletal muscle; 2) altered endothelium-dependent vasoregulatory responses; 3) skeletal muscle contractile dysfunction; and 4) the development of capillary no-reflow and myocyte necrosis in postsischemic skeletal muscle and heart (7, 8, 13). Once adherent to endothelium, neutrophils undergo further activation during extravasation to produce inflammatory tissue damage. Although it is clear that activated leukocytes play a major role in the pathogenesis of I/R, the mechanisms whereby these cells are recruited to target tissues are not completely understood. It has been suggested that reactive oxygen species from both target tissue and leukocyte contribute to the full development of the postsischemic inflammatory response. For example, brain injury arising secondary to transient middle cerebral artery occlusion was reduced in mice lacking a functional NADPH oxidase, relative to their wild-type littermates (20). Knockout of both neuronal and myeloid sources of NADPH oxidase activity was required for significant protection against reperfusion injury. Regardless of the exact mechanisms involved, therapies designed to inhibit leukocyte adhesion to activated endothelium are of potential use as anti-inflammatory agents.

In these studies, I/R was associated with a marked and progressive increase in leukocyte adhesion to and emigration across single postcapillary venules in the rat mesentery. The postsischemic leukosequestration was temporally associated with increased oxidant production, as detected by an increased fluorescence of the oxidant-sensitive probe DHR, and microvascular barrier disruption, as indicated by the increased leakage of fluorescently labeled albumin across postcapillary venules. Our results indicate that PR-39 completely abolished the increase in postsischemic oxidant production and VPL. The reduced oxidant production noted in the postsischemic rat mesentery is consistent with our observations that PR-39 reduced superoxide production by activated rat neutrophils in vitro.

An unexpected finding of the present study was that PR-39 completely abolished leukocyte rolling, stationary adherence, and emigration in postsischemic mesenteric venules and prevented PMA-induced leukocyte adhesion to endothelial cell monolayers. We also observed that PR-39 prevented PMA-induced ICAM-1 expression in cultured endothelial cells. Interestingly, no emigrated leukocytes were observed in the mesentery of PR-39-treated rats during basal conditions. The latter observation suggests that PR-39 may influence some constitutive mechanism involved in neutrophil trafficking. It is unlikely that PR-39 suppression of...
basal ICAM-1 expression by venular endothelium is responsible for this observation, because PR-39 treatment of non-PMA-treated cultured endothelial cells only marginally affected ICAM-1 levels (Fig. 2A).

The mechanism(s) underlying the effect of PR-39 to block postischemic leukocyte adhesion and emigration is unclear, because this peptide interacts with several potentially relevant pathways operative in the proinflammatory cascade. Our earlier work suggested that PR-39 blocked NADPH oxidase activity in human and pig neutrophils by interfering with the interaction of the p22phox and p47phox subunits during oxidase assembly. Subsequently, Chan and Gallo (3) have demonstrated that PR-39 can modulate the behavior of at least one other Src homology-3 domain-containing cell regulatory protein, p130cas. Thus the possibility that multiple potential targets exist for PR-39 makes its mechanism of action difficult to define, particularly in the complex inflammatory environment.

In addition to its demonstrated antioxidant effect on neutrophils, PR-39 blocks the activity of an NADPH oxidase-like enzyme in cultured endothelial cells (Ref. 2; A. Barchowsky, Dartmouth Medical School; J. Kefer, University of Illinois College of Medicine, personal communication). Several investigators (2, 10) have demonstrated the presence of oxidase components in endothelial cells, including gp91phox, p22phox, p67phox, and p47phox. Although the regulation and functional significance of this endothelial enzyme has not been established, the possibility that it plays a role in initiating or amplifying the I/R cascade should be considered. To address this possibility, we measured DHR oxidation after 10 min of reperfusion, a time point that precedes the establishment of postischemic leukocyte-endothelial interactions and which is thought to represent a time frame over which oxidant-dependent proinflammatory stimuli are first elaborated (14). However, we noted no difference in oxidant production, as detected by DHR oxidation, in mesenteries subjected to I/R alone or I/R + PR-39 at this time point. These observations suggest that oxidant production involved in the elaboration of chemotactic stimuli in the mesentery may occur at a level below that which we are able to detect with the DHR approach. However, our data are most supportive of the possibility that PR-39 prevents oxidant production and tissue damage by preventing neutrophil recruitment or that oxidants derived from the NADPH oxidase of adherent neutrophils generate proinflammatory signals that amplify leukocyte-endothelial interactions and which is thought to represent a time frame over which oxidant-dependent proinflammatory stimuli are first elaborated (14).

Support for this possibility is provided by our observation that PR-39 prevented PMA-induced ICAM-1 expression in cultured endothelial cells. This latter observation is consistent with the observation that PR-39 prevents oxidant-associated nuclear translocation of the transcription factor NF-κB (A. Barchowsky and J. Kefer, personal communication). Because NF-κB translocation has been shown to be a requisite step in the expression of functional adhesion molecules on the endothelium (4, 12), it is possible that PR-39 may prevent adhesion at later time points in reperfusion by this mechanism, in addition to its direct effects on the neutrophil NADPH oxidase.

An additional explanation for the effect of PR-39 to block leukocyte adhesion is provided by the results obtained when we evaluated PAF-induced neutrophil chemotaxis in the presence of PR-39. Although both PAF and PR-39 are neutrophil chemoattractants when used alone (10), when tested in combination, the chemotactic response was blunted. Others have observed similar neutrophil responses to mixtures of chemotactic stimuli, in a process termed heterologous desensitization (11). It is possible then that PR-39 blocks an early proinflammatory step in the postischemic period, perhaps involving PAF, by a pathway that is independent of neutrophil oxidant production.

In summary, the results of this study indicate that PR-39 completely abolishes postischemic leukocyte adhesion and emigration. The mechanisms underlying this observation remain to be elucidated but may be traced to inhibition of cellular oxidant production, adhesion molecule expression, or neutrophil chemotaxis. PR-39 may thus be therapeutically useful for prevention of neutrophil adhesion and activation during the postischemic inflammatory response.

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