Reduction in Reperfusion-Induced Myocardial Necrosis in Dogs by RheothRx Injection (Poloxamer 188 N.F.), a Hemorheological Agent That Alters Neutrophil Function

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Background Reperfusion after prolonged coronary artery occlusion may be followed by additional myocardial necrosis persisting for hours to days. Potential mechanisms include neutrophil-mediated injury and compromised flow within the microcirculation of the reperfused myocardium. Poloxamer 188 is a nonionic surfactant with beneficial hemorrheological and neutrophil-inhibitory properties. The purpose of the present study was to determine if poloxamer 188 is capable of reducing the myocardial injury associated with sustained reperfusion and to examine the effect of treatment duration.

Methods and Results Three groups of closed-chest dogs underwent 90 minutes of left anterior descending coronary artery occlusion (angioplasty balloon) and 72 hours of reperfusion. Poloxamer 188, formulated as RheothRx Injection (Burroughs Wellcome Co), was given at a dose of 75 mg/kg IV bolus 15 minutes before reperfusion followed by a continuous IV infusion for 4 hours (n=13) or 48 hours (n=13); control dogs (n=12) received saline for 48 hours. The 48-hour infusion of poloxamer 188 resulted in a 42% reduction in infarct size (as a percent of the area at risk) compared with the control group (25.0±4.2% versus 43.3±4.3%, P<.01), whereas the 4-hour group demonstrated a 25% reduction in infarct size compared with the control group (32.4±4.3%, P=.08). ANCOVA demonstrated that the 48-hour infusion of poloxamer 188 reduced myocardial infarct size independent of differences in collateral blood flow (P=.002 versus control). A trend toward infarct size reduction was observed in the 4-hour infusion group (P=.098 versus control by ANCOVA). Plasma creatine phosphokinase concentration was lower in both poloxamer 188-treated groups (P<.05 versus control). Global left ventricular ejection fraction at 72 hours of reperfusion was improved in the 48-hour infusion group compared with the control group (43±3.1% versus 33±2.0%, P<.05), whereas ejection fraction in the 4-hour group was 37±1.3% (P=NS versus control). Regional ventricular function was also significantly better in the 48-hour infusion group compared with the control group. In vitro studies demonstrated that at concentrations comparable to those achieved in vivo, poloxamer 188 inhibited neutrophil chemotaxis. This finding may represent a beneficial mechanism of action.

Conclusions A 48-hour infusion of poloxamer 188 reduced myocardial infarct size and improved left ventricular function in this dog model of 90 minutes of coronary artery occlusion and 72 hours of reperfusion. The finding that the 4-hour infusion of poloxamer 188 did not result in similar benefits suggests that additional reperfusion injury occurred between 4 and 48 hours. (Circulation. 1994;90:2964-2975.)

Key Words • infarction • reperfusion • neutrophils • poloxamer 188

Although thrombolytic therapy1,2 and primary percutaneous transluminal coronary angioplasty (PTCA)3-4 are established as effective therapies for acute myocardial infarction, adverse clinical events (eg, reinfarction, heart failure, death) continue to occur. In an attempt to further reduce morbidity and mortality, attention has shifted toward identifying agents that may be administered concurrently with reperfusion therapy. A new class of agents that may prove to be useful adjuncts to thrombolytic therapy or PTCA are drugs that reduce myocardial injury caused by reperfusion itself.

Reperfusion-induced myocardial necrosis is a form of reperfusion injury that has been documented in numerous experimental studies.5-9 An important mechanism responsible for this event apparently involves an inflammatory response that rapidly develops within the reperfused myocardium.10 Neutrophils accumulate during the early hours of reperfusion,11,12 attracted by complement components,13 leukotriene B4,14,15 and other cytokines.16 Stimulated neutrophils can then injure viable myocytes and vascular endothelial cells by releasing toxic oxygen-derived free radicals or proteolytic enzymes.17 Another mechanism involves clumping and sludging of neutrophils and other cellular elements in the newly reperfused microcirculation, a process known as the “no-reflow” phenomenon.18-20 This event deprives nutritive flow to regions of the myocardium and results in additional cell death despite restoration of epicardial patency.
Poloxamer 188 is a nonionic block copolymer surfactant that has several actions that might be expected to ameliorate reperfusion injury. First, poloxamer 188 has beneficial hemorheological properties caused by its ability to coat cellular elements (neutrophils, platelets, endothelial cells), thereby reducing the surface tension between cells and decreasing their adhesiveness.21 Second, poloxamer 188 has been shown to inhibit neutrophil chemotaxis and adhesion in vitro.22 In an open-chest canine model of 90 minutes of coronary artery occlusion and 24 hours of reperfusion, administration of poloxamer 188 was associated with a reduction in myocardial infarct size.23 In addition, poloxamer 188 is an important component of the oxygen-carrying perfluorochemical emulsion Fluosol, where it serves as an emulsifying agent comprising 2.7% w/v of the emulsion. Fluosol has been shown to reduce reperfusion injury in dogs24,25,26 and rabbits27 and in a pilot study of patients undergoing PTCA for acute myocardial infarction.28

The purpose of the present study was to evaluate the effects of poloxamer 188 on infarct size and left ventricular function in a closed-chest canine model of 90 minutes of coronary artery occlusion and 72 hours of reperfusion. Because prior work suggested that additional reperfusion-induced myocardial injury can occur up to 48 hours after reperfusion,29 we also sought to clarify the most effective duration of treatment with this agent. Thus, we used two dosing regimens of poloxamer 188 during reperfusion: a short, 4-hour infusion and a longer, 48-hour infusion.

Methods

The present study conformed to the guidelines specified in the National Institutes of Health “Guide for Care and Use of Laboratory Animals” (NIH publication no. 86-23, revised 1985) and was approved by the Institutional Animal Care and Use Committee of Rush Medical College.

Surgical Procedures

Healthy adult male mongrel dogs (20 to 30 kg) were anesthetized with sodium pentobarbital (15 to 20 mg/kg IV), endotracheally intubated, and mechanically ventilated with room air. The arterial pH and Pco₂ were maintained at 7.35 to 7.42 and 35 to 45 mm Hg, respectively. Supplemental doses of pentobarbital were given as needed to maintain anesthesia. The ECG was continuously monitored in leads II and V₃.

All surgical procedures were performed using aseptic technique. Animals were positioned supine on the catheterization table, and bilateral femoral cutdowns were performed. An 8F introducer sheath (Cordis Corp) was inserted into the left femoral artery. Through this sheath, a 7F micromanometer-tipped pigtail catheter (model SPC474A, Millar Instruments) was advanced to the apex of the left ventricle for continuous monitoring of left ventricular pressure, injection of microspheres, and for performance of contrast ventriculography. A 9F sheath was inserted into the right femoral artery, through which an 8F “hockey stick” angioplasty guiding catheter (Advanced Cardiovascular Systems) was used to cannulate the left main coronary artery. The right external jugular vein was isolated by cutdown, and a 7F double-lumen catheter (Cordis Corp) was inserted into the superior vena cava for subsequent poloxamer 188 or saline infusions and for obtaining blood samples. For long-term placement, the venous catheter was tunneled subcutaneously and exteriorized through the interscapular region.

After placement of catheters, 3000 IU heparin IV (derived from porcine intestines, Elkins-Sinn Inc) was given. A 0.014-in guide wire (Advanced Cardiovascular Systems) was advanced through the angioplasty guide and positioned in the distal left anterior descending coronary artery (LAD). An angioplasty catheter (3.0 mm balloon diameter, 20 mm length; Advanced Cardiovascular Systems) was advanced over the guide wire and positioned so that the proximal portion of the balloon was immediately distal to the first major diagonal branch. Coronary artery occlusion was produced by inflating the balloon to 4 atm of pressure, and total occlusion of the LAD was confirmed by coronary angiography. The precise location of the balloon relative to angiographic landmarks was carefully noted. After 90 minutes of LAD occlusion, the balloon was deflated, and the catheter was removed. Animals developing ventricular fibrillation during occlusion or reperfusion were defibrillated with 200 J. A maximum of three defibrillation attempts were made for each individual episode. Lidocaine was not administered in this study because it has been previously shown to reduce myocardial infarct size after reperfusion in a canine model,30 a benefit that may involve inhibition of neutrophil function.31,32

After 2 hours of reperfusion, catheters and sheaths were removed, the femoral cutdown sites were surgically repaired, and the animal was fitted with a jacket (part no. 012-1890, Alice King Chatham Medical Arts) specially designed to reduce the chance of dislodgement of the chronically implanted jugular venous catheter. Each animal was then transferred to a large, individual cage fitted with an infusion pump (IMED Corp) that permitted continuous delivery of poloxamer 188 or saline (in the control group) from an external reservoir into the jugular venous catheter via polyethylene tubing. The tubing was channeled through a flexible stainless-steel tether (part no. 312-0030, Alice King Chatham), one end of which was secured to the jacket while the other end was connected to the infusion pump through a swivel (part no. 070-0020, Alice King Chatham) mounted at the top center of the cage. This apparatus permitted the dogs to move freely within the cage, while reducing the likelihood of interruption of the infusion.

At 72 hours of reperfusion, dogs were returned to the catheterization laboratory. General anesthesia and mechanical ventilation were performed as above. An 8F introducer sheath was inserted into the right femoral artery, which was exposed by surgical cutdown. With a 7F 3.5 left Judkins angiographic catheter (Cordis Corp), left coronary angiography was performed to confirm patency of the LAD. After removal of the angiographic catheter, the 7F micromanometer-tipped pigtail catheter was placed into the left ventricle, and hemodynamic measurements and left ventriculography were performed. Dogs were then euthanatized with a lethal dose of intravenous potassium chloride, and the heart was excised.

Dosing Protocol

Poloxamer 188 was given as RheothRx Injection (Burroughs Wellcome Co), a sterile solution containing 140.4 mg/mL poloxamer 188, 4.5 mg/mL NaCl, and 1.8 µg/mL butylated hydroxytoluene. Dogs were randomly assigned to one of three groups. One group received an intravenous bolus of poloxamer 188 at 75 mg/kg, 15 minutes before reperfusion. This was immediately followed by a continuous intravenous infusion of poloxamer 188 at 150 mg·kg⁻¹·h⁻¹ for 4 hours (rate, 1.07 mL·kg⁻¹·h⁻¹), after which the infusion was changed to half-normal sterile saline (4.5 mg/mL) given at the same rate for the subsequent 44 hours. The infusion was then discontinued for the final 24 hours of reperfusion. The administration of poloxamer 188 shortly before reperfusion simulates its use as an adjunct to intravenous thrombolytic therapy in the clinical management of acute myocardial infarction. To provide sustained treatment with poloxamer 188 beyond the early reperfusion period, a second group of dogs received the same bolus of poloxamer 188 as above 15 minutes before reperfusion, followed by a continuous intravenous infusion at 150
mg·kg⁻¹·h⁻¹ for 48 hours (same rate as above). The infusion was discontinued for the final 24 hours of reperfusion. Thus, a 25-kg dog received a 13.35-mL bolus of poloxamer 188 followed by 26.71 mL/h for either 4 or 48 hours. The third group (control) received a sterile saline solution (4.5 mg/mL) at 1.07 mL·h⁻¹·kg⁻¹ for 48 hours.

**Hemodynamic Measurements**

The analog signals from the micromanometer and ECG were passed through signal processors (Hewlett Packard) and recorded with calibration signals on a thermal paperwriter (Gould Electronics, Inc). Heart rate, left ventricular peak systolic pressure, and left ventricular end-diastolic pressure were collected at baseline (before LAD occlusion), at 60 minutes of coronary artery occlusion, and at 2 and 72 hours after reperfusion. Rate-pressure product was calculated as the product of heart rate and left ventricular peak systolic pressure.

**Assessment of Left Ventricular Function**

To assess left ventricular function, contrast ventriculography was performed at baseline before LAD occlusion, at 60 minutes of occlusion, and at 2 and 72 hours after reperfusion. Ventriculography was performed using 0.50 to 0.75 mL/kg iotinated contrast (Renografin-76, Squibb Diagnostics), power injected at 10 to 14 mL/s (Angioman 6000, Liebel-Flarsheim Company) and recorded on cine film at 60 frames per second in a 30-degree right anterior oblique projection.

Ventriculograms were analyzed by a collaborator blinded to the treatment group. End-diastolic and end-systolic images from the cine film were digitized using an image analysis system (ImageComm Systems, Inc), and the ventricular silhouettes were traced. Beats following premature ventricular contractions were excluded.

Global left ventricular ejection fraction was calculated using the Sandler-Dodge formula. Regional wall motion was assessed using the centerline method. Briefly, this method uses 100 equally spaced chords constructed perpendicularly to a centerline drawn midway between the end-diastolic and end-systolic contours. The length of a given chord represents the wall motion of the corresponding point on the ventricular contour. Adjustment for heart size is made by dividing each chord length by the end-diastolic perimeter length, producing 100 unitless shortening fractions. Although clinical studies express the fractional shortening for a given chord in units of standard deviations from the mean motion of that same chord from a database of patients with normal left ventricular function, the present study included acquisition of a baseline (preinfarction) ventriculogram. This permitted comparison of regional wall motion during occlusion and after reperfusion to the baseline function for each animal. The LAD territory was defined as chords 12 to 66, as previously demonstrated in a canine model of myocardial infarction and reperfusion.

**Myocardial Blood Flow Determinations**

Myocardial blood flow was assessed using 15-μm colored polystyrene microspheres (orange, black, red, or green; EZ Trac) with determinations at baseline (before LAD occlusion), at 1 hour of LAD occlusion (to assess collateral blood flow), and at 1 and 2 hours after reperfusion. The colored-microsphere technique has been previously validated with methods described in detail. Microspheres (5 to 8 x 10⁶) were mixed by vortex agitation for 1 minute and injected through the central lumen of the pigtail catheter into the apex of the left ventricle. Microsphere injections into the left ventricular apex have been shown to compare favorably with left atrial injections. A withdrawal pump (Harvard Apparatus) was connected to the sideport of the 8F left femoral arterial sheath, and reference blood was withdrawn at 15.3 mL/min, beginning 5 seconds before each microsphere injection and continuing for 90 seconds after each injection. Determination of myocardial blood flow and collection of hemodynamic data always preceded contrast ventriculography to avoid the potentially confounding effect of contrast on these variables.

For the determination of myocardial blood flow, 1- to 2-g samples of myocardial tissue were cut from epicardial and endocardial halves obtained from the central 50% of the risk area and from the nonischemic posterior wall. Reference blood and myocardial tissue samples were processed with digestive reagents by methods previously described. Final aliquots from the blood and tissue samples containing the microspheres were counted with a Fuchs-Rosenthal hemocytometer, and four to eight chambers were counted for each sample. All counting was performed by an observer blinded to the treatment group.

Myocardial blood flow (MBF, in mL·min⁻¹·g⁻¹) was calculated from the formula: MBF=(Cm×Qr)/(Cr×WT), where Cm is the total number of microspheres counted in the myocardial tissue sample, Qr is the rate of reference blood withdrawal (mL/min), Cr is the total number of microspheres counted in the reference blood sample, and WT is the weight of the tissue sample (g).

**Poloxamer 188 Plasma Concentrations**

For determination of plasma concentrations of poloxamer 188, 5 mL of blood was drawn from the jugular venous catheter (see "Surgical Procedures") into EDTA-containing Vacutainers at the following intervals: before LAD occlusion and at 1, 2, 4, and 6 hours of reperfusion. Dogs in the poloxamer 188 48-hour infusion group had additional samples drawn at 24 and 48 hours of reperfusion. To assess the rate of elimination of poloxamer 188 after its discontinuation, additional blood samples were drawn at 10, 20, 40, 60, and 120 minutes after termination of poloxamer 188 infusion in both poloxamer 188 groups. An additional sample at 240 minutes after drug termination was drawn in the poloxamer 188 48-hour infusion group.

Plasma samples for determination of poloxamer 188 concentration were subjected to protein precipitation and were analyzed by gel permeation chromatography (GPC) with detection by differential refractive index. Calibration standards of poloxamer 188 (0.05 to 2.0 mg/mL) were prepared in duplicate. The ratio of the peak area of poloxamer 188 to the peak area of the internal standard was calculated. A least-squares regression of the natural log of these ratios on their respective concentrations was generated. The poloxamer 188 concentration of each plasma sample was calculated by fitting the sample natural log ratios to the regression model.

**In Vitro Neutrophil Function Studies**

Neutrophils isolated from a separate group of healthy dogs that did not undergo the infarction protocol were used to determine the effect of poloxamer 188 on in vitro neutrophil chemotaxis (n=7 dogs) and superoxide anion release (n=6 dogs). Venous blood was collected into heparinized tubes, and neutrophils were isolated by a 30-minute density gradient centrifugation in Histopaque (Sigma Chemical Co) followed by a 20-minute dextran sedimentation. To rid the neutrophil pellet of any remaining red blood cells, we resuspended samples in 0.2% saline for 20 seconds followed by 1.6% saline for 20 seconds. Samples were spun a final time and resuspended in Hanks' buffered salt solution with calcium (HBSS; Gibco BRL). Final cell concentration was adjusted to 10⁶ cells/mL. Neutrophil viability was demonstrated by trypan blue exclusion and confirmed to be >95%.

Neutrophil chemotaxis was assessed using a 48-well modified Boyden chamber with 5% zymosan (Sigma Z4250)-acti-vated serum as the chemotactrant. Neutrophils (10⁶) were incubated for 35 minutes in wells containing 0.25, 0.50, 1.0, and 2.0 mg/mL of poloxamer 188. Incubation with HBSS alone served as the control. Four wells were studied for each of the five conditions. After the incubation, the polycarbonate filters (Neuro Probe Inc) were removed and stained with Wright's.
stain. Chemotaxis was quantified by counting the number of neutrophils that migrated across the polycarbonate filter. For each well, five random high-power fields (×100 oil immersion) were counted and averaged. The mean neutrophil count at each concentration was obtained by averaging the results from all wells.

Neutrophil superoxide anion release was assessed as the superoxide dismutase inhibitable reduction of ferriytochrome C (80 μmol/L, Sigma). The cells (10^6/mL) were incubated at 37°C for 30 minutes in the presence of the following concentrations of poloxamer 188: 0.01, 0.03, 0.10, 0.30, 1.0, 3.0, and 10.0 mg/mL. Cells incubated in HBSS alone served as the control for spontaneous release of superoxide anion. Reduced cytochrome C was quantitated by measuring its optical density at 550 nm.

**Quantitation of Myocardial Infarct Size**

To determine the quantity of infarcted myocardium as well as the size of the myocardial bed at risk to infarction, an ex vivo dual-perfusion histochemical staining technique was used.30 The triphenyl tetrazolium chloride (TTC) technique used to quantitate myocardial infarct size in this study has been previously shown to compare favorably with histopathologic measurements of infarct size in our laboratory25 and by others.39-40 Polyethylene catheters of equal internal diameter were used to cannulate the aortic root above the coronary ostia and the LAD just beyond the site of previous balloon occlusion. The LAD was then ligated immediately distal to the first major diagonal branch (site of balloon occlusion). The LAD distal to the ligature was perfused with sodium phosphate buffer (pH 8.4, 37°C) containing 1.5% TTC. The aorta was simultaneously perfused in a retrograde manner with 1.5% blue dye (Unisperse Blue, CIBA-Geigy Corp.). These solutions were infused at equal pressures (120 mm Hg) for 5 minutes while the heart was suspended in a 37°C water bath.

After staining, the heart was cut from apex to base into 1-cm slices parallel to the atroventricular groove. The left ventricle (including the interventricular septum) was then separated from the remainder of the heart. In this manner, the area at risk to infarction (previously perfused by the LAD distal to the site of occlusion) was identified by the absence of blue staining. Within the area at risk, viable myocardium stains deep red (TTC stains viable myocardium containing dehydrogenase enzymes), whereas regions of infarct appear pale yellow (necrotic tissue depleted of dehydrogenase enzymes), whereas regions of infarct appear pale yellow (necrotic tissue depleted of dehydrogenase enzymes) that is not stained by TTC. Each slice was weighed, and its basal surface was photographed. The areas at risk and areas of infarction were then traced from enlarged (×3) projections of the photographs by an observer blinded to the treatment group. Computer-aided planimetry was used to quantitate the area at risk (expressed as a percentage of the total left ventricle) and the area of infarction (expressed as both a percentage of the area at risk and a percentage of the total left ventricle).

**Creatine Phosphokinase and Hematologic Assessment**

Aliquots of plasma were prepared from the same blood samples collected for the determination of poloxamer 188 plasma concentrations (before coronary artery occlusion and at 1, 2, 4, 6, and 24 hours of reperfusion). Creatine phosphokinase (CPK) levels were determined using a commercially available kit (520, Sigma Chemical Co) in which phosphocreatine is converted by CPK to creatine, which, in the presence of α-naphthol and diacetyl, forms a colored complex measured at 520 nm. CPK was quantitated from a standard curve generated using known amounts of creatine. The addition of authentic poloxamer 188 (0.5 to 20 mg/mL) to creatine standard in blank plasma did not interfere with the assay. Results represent the mean of two or more assays per sample, which differed by no more than 10%.

Hematocrit, white blood cell count, and neutrophil count were measured in each animal before coronary artery occlusion, at 60 minutes after occlusion, and at 3, 24, and 72 hours after reperfusion.

**Light Microscopy**

To evaluate the effect of poloxamer 188 treatment on the extent of inflammatory infiltrate after 72 hours of reperfusion, transmural myocardial tissue from the central ischemic region was analyzed by light microscopy. Myocardial tissue from the full circumference of a midventricular slice was fixed in 1% glutaraldehyde/4% formaldehyde, cut at 6 μm, and stained with Masson's trichrome and hematoxylin and eosin. The slides were evaluated by an observer blinded to the treatment group. A semiquantitative analysis of the degree of neutrophil and macrophage infiltration, hemorrhage, and endothelial edema was performed according to the method of Romson et al.41 Specimens were graded on a scale of 0 to 4, with 0 representing no infiltration, hemorrhage, or endothelial edema, and 4 representing extensive infiltration, hemorrhage, or endothelial edema.

**Statistical Analysis**

Data are presented as mean±SEM values. Comparison of infarct size and area at risk between treated groups and control was made using Student's t test for unpaired data (two-tailed). Because collateral blood flow is a critical determinant of myocardial infarct size and is highly variable in dogs, ANCOVA was performed to examine the effect of treatment on infarct size (the dependent variable), while controlling for differences in collateral blood flow (the independent variable). For myocardial blood flow, left ventricular ejection fraction, regional wall motion, hemodynamic, and hematologic data, two-way repeated measures ANOVA was used to assess differences among the groups and within groups. When ANOVA indicated the presence of significant differences, a multiple comparison procedure (Tukey's test) was used to determine the time points at which the groups differed. One-way ANOVA, followed by Student's t test (unpaired), was used to assess the differences among the three groups with respect to postischemic release of CPK (determined as area under the CFP versus reperfusion time curve). The effect of different concentrations of poloxamer 188 on neutrophil release of superoxide anion was analyzed using Student's t test with Bonferroni's correction for multiple comparisons. One-way ANOVA was performed to examine the effect of different concentrations of poloxamer 188 in vitro neutrophil chemotaxis. Differences among the groups with respect to incidence of ventricular fibrillation were assessed using Fisher's exact test. For all tests, a value of P<.05 was considered significant.

**Results**

**Study Groups**

Fifty-four dogs were entered into the study and randomized into one of the three study groups (Table 1). Thirty-eight dogs were included in the final analysis. Ten dogs were excluded because of refractory ventricular fibrillation during coronary artery occlusion (before poloxamer 188 or saline administration, n=9) or after reperfusion (n=1). Three animals died in the subsequent 18 hours after occlusion. An additional 3 dogs in the poloxamer 188 48-hour group were excluded because of an early, prolonged interruption in the poloxamer 188 infusion, confirmed by a substantial reduction in poloxamer 188 plasma concentration (data not shown).
Incidence of Ventricular Fibrillation

During coronary artery occlusion (before poloxamer 188 or saline administration), the incidence of ventricular fibrillation did not differ among the three groups (P = NS), averaging 35%. Successful defibrillation was also not different among the three groups during coronary artery occlusion (P = NS), averaging 53%. Similarly, there was no significant difference in the incidence of ventricular fibrillation among the groups during the initial 3 hours of reperfusion: 36% (5 of 14) in the control group, 54% (7 of 13) in the poloxamer 188 4-hour group, and 39% (7 of 18) in the poloxamer 188 48-hour group. Defibrillation was successful in all poloxamer 188–treated dogs and in 4 of 5 control dogs (P = NS).

Hematologic Data and Arterial Blood Gases

In all three groups, a similar significant increase in total white blood cell count and neutrophil count was observed 24 hours after reperfusion compared with baseline (Table 2). No significant differences in hematocrit were observed among the three groups, although the 48-hour infusion group demonstrated a reduction in hematocrit from baseline to 72 hours after reperfusion that reached statistical significance (P < .05). No significant differences in arterial blood gases were seen among the three groups, either at baseline (preocclusion), during coronary artery occlusion, or 1 or 72 hours after reperfusion. pH, PO₂, and PCO₂ values remained within the physiological range throughout the experiment, and there was no requirement for supplemental oxygen.

Hemodynamic Effects

Compared with control dogs, administration of poloxamer 188 for 4 or 48 hours after reperfusion had no significant effect on heart rate, left ventricular peak systolic pressure, or left ventricular end-diastolic pressure (Table 3). During coronary artery occlusion, there were no differences among the groups in rate-pressure product (an index of myocardial oxygen consumption and determinant of myocardial infarct size).

Poloxamer 188 Plasma Levels

In the 48-hour infusion group, the 75 mg/kg IV bolus of poloxamer 188 followed by the continuous infusion of 150 mg·kg⁻¹·h⁻¹ for 48 hours resulted in sustained poloxamer 188 plasma concentrations of 1.2±0.1 mg/mL at 1 hour, 1.5±0.2 mg/mL at 4 hours, 1.2±0.1 mg/mL at 24 hours, and 1.4±0.2 mg/mL at 48 hours (Fig 1). After discontinuation of the poloxamer 188 infusion, the compound was rapidly cleared from the circulation.

Poloxamer 188 plasma concentrations in the 4-hour infusion group (given an identical bolus and infusion dose as above) were not significantly different from the 48-hour infusion group and had poloxamer 188 plasma concentrations of 1.0±0.1 at 1 hour and 1.3±0.1 at 4 hours. As in the 48-hour infusion group, poloxamer 188 was rapidly cleared after discontinuation of the infusion.

### Table 1. Dogs Entered in and Excluded From the Study

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>P-188 4-h</th>
<th>P-188 48-h</th>
<th>Total</th>
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<tbody>
<tr>
<td>Dogs entered, n</td>
<td>17</td>
<td>15</td>
<td>22</td>
<td>54</td>
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<tr>
<td>Dogs excluded, n</td>
<td></td>
<td></td>
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<tr>
<td>Died (VF coronary occlusion)</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>9</td>
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<tr>
<td>Died (VF reperfusion 0-3 h)</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>Died (3-18 h after reperfusion)</td>
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<td>0</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Infusion interrupted</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Total excluded</td>
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<td>9</td>
<td>16</td>
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<tr>
<td>Included in final analysis, n</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>38</td>
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P-188 4-h indicates poloxamer 188 4-hour infusion group; P-188 48-h, poloxamer 188 48-hour infusion group; and VF, ventricular fibrillation.

### Table 2. Hematologic Data in Control and Treated Groups

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<tr>
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<th>Control (n=12)</th>
<th>P-188 4-h (n=13)</th>
<th>P-188 48-h (n=13)</th>
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<td>WBC, ×10³/L</td>
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<td>RP-24 h</td>
<td>27.7±2.3*</td>
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<td>16.3±2.2</td>
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<td>PMNs, ×10³/L</td>
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<td>Baseline</td>
<td>9.2±1.7</td>
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<td>8.8±0.9</td>
</tr>
<tr>
<td>OC-1 h</td>
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<td>10.4±1.5</td>
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<td>42±2</td>
<td>40±2</td>
<td>37±1</td>
</tr>
<tr>
<td>RP-24 h</td>
<td>36±3</td>
<td>38±2</td>
<td>35±2</td>
</tr>
<tr>
<td>RP-72 h</td>
<td>33±3</td>
<td>33±2</td>
<td>27±5*</td>
</tr>
</tbody>
</table>

P-188 4-h indicates poloxamer 188 4-hour infusion group; P-188 48-h, poloxamer 188 48-hour infusion group; WBC, white blood count; OC, coronary artery occlusion; RP, reperfusion; and PMNs, neutrophil count. Values are given as mean±SEM. *P < .05 vs baseline; no significant differences among groups (by ANOVA).
TABLE 3. Hemodynamic Effects of Poloxamer 188 in Control and Treated Groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>P-188 4-h (n=13)</th>
<th>P-188 48-h (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>138±8</td>
<td>138±6</td>
<td>142±7</td>
</tr>
<tr>
<td>OC-1 h</td>
<td>136±6</td>
<td>141±5</td>
<td>139±5</td>
</tr>
<tr>
<td>RP-2 h</td>
<td>133±7</td>
<td>140±8</td>
<td>128±7</td>
</tr>
<tr>
<td>RP-72 h</td>
<td>145±6</td>
<td>138±4</td>
<td>147±7</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>155±3</td>
<td>152±5</td>
<td>144±3</td>
</tr>
<tr>
<td>OC-1 h</td>
<td>129±7*</td>
<td>137±6</td>
<td>132±6</td>
</tr>
<tr>
<td>RP-2 h</td>
<td>123±6*</td>
<td>135±7</td>
<td>127±7</td>
</tr>
<tr>
<td>RP-72 h</td>
<td>144±5</td>
<td>128±6*</td>
<td>136±6</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.8±1.3</td>
<td>5.0±1.0</td>
<td>6.3±1.0</td>
</tr>
<tr>
<td>OC-1 h</td>
<td>4.9±1.9</td>
<td>5.6±1.5</td>
<td>5.0±1.4</td>
</tr>
<tr>
<td>RP-2 h</td>
<td>2.4±1.2</td>
<td>4.9±1.4</td>
<td>4.4±1.2</td>
</tr>
<tr>
<td>RP-72 h</td>
<td>10.7±2.0</td>
<td>6.6±1.1</td>
<td>9.3±1.7</td>
</tr>
<tr>
<td>RPP, bpm·mm Hg⁻¹·h⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21.3±1.2</td>
<td>21.0±1.2</td>
<td>20.4±1.1</td>
</tr>
<tr>
<td>OC-1 h</td>
<td>18.0±1.7</td>
<td>19.6±1.4</td>
<td>18.3±1.1</td>
</tr>
<tr>
<td>RP-2 h</td>
<td>16.7±1.6</td>
<td>19.4±2.0</td>
<td>16.5±1.6</td>
</tr>
<tr>
<td>RP-72 h</td>
<td>21.1±1.4</td>
<td>17.3±1.4</td>
<td>20.2±1.6</td>
</tr>
</tbody>
</table>

P-188 4-h indicates poloxamer 188 4-hour infusion group; P-188 48-h, poloxamer 188 48-hour infusion group; OC, coronary artery occlusion; RP, reperfusion; bpm, beats per minute; LVSP, left ventricular peak systolic pressure; LVEDP, left ventricular end-diastolic pressure; and RPP, rate-pressure product (the product of heart rate and LVSP). Values are given as mean±SEM. *P<.05 vs baseline; no significant differences among groups (by ANOVA).

Regional Myocardial Blood Flow

No significant differences in endocardial and epicardial (Table 4) or transmural blood (Fig 2) flows were observed among the three study groups at any time during the experiment (P=NS by ANOVA). Importantly, during coronary artery occlusion, transmural myocardial blood flow fell markedly (P<.01 compared with baseline) and to a similar extent in each group, indicating similar collateral blood flows. Myocardial blood flow returned to preocclusion values after reperfusion at both 1 and 2 hours in each of the three groups. Endocardial and epicardial blood flows to the nonischemic circumflex region (Table 4) were similar among the three groups at all time points measured during the experiment (P=NS by ANOVA).

Myocardial Infarct Size

The effect of poloxamer 188 on myocardial infarct size is shown in Fig 3. The area at risk to infarction was similar among the three groups (P=NS), averaging 30.4±1.2% of the left ventricle. The poloxamer 188 48-hour infusion group demonstrated a 42% reduction in infarct size (expressed as percent of the area at risk) compared with the control group (25.0±4.2% versus 43.3±4.3%, P<.01). The 48-hour infusion group also showed a significant reduction in infarct size when expressed as percent of the total left ventricle compared with control (8.1±1.7% versus 13.3±1.7%, P<.05).

The 4-hour infusion group demonstrated a 25% reduction in infarct size (expressed as percent of the area at risk) compared with the control group, which did not reach significance (32.4±4.3% versus 43.3±4.3%, P=.08). No significant difference was observed when infarct size was expressed as a percent of the left ventricle (P=.17).

Relation of Myocardial Infarct Size to Collateral Blood Flow

To control for the marked variability in collateral blood flow among dogs (a critical determinant of myocardial infarct size), the relation between transmural collateral flow and infarct size (expressed as a percent of the area at risk) was plotted (Fig 4). All three groups demonstrated significant inverse correlations between infarct size and transmural collateral blood flow: control group (r=-.83, P=.0007), poloxamer 188 4-hour infusion group (r=-.63, P=.02), and poloxamer 188 48-hour infusion group (r=-.68, P=.01). ANCOVA demonstrated that treatment with poloxamer 188 for 48 hours significantly reduced myocardial infarct size compared with controls (P=.002) and that this infarct size reduction was independent of differences in collateral blood flow. The 4-hour infusion of poloxamer 188 also resulted in a trend toward infarct size reduction compared with the control group (P=.098 by ANCOVA).

CPK Release During the Initial 24 Hours of Reperfusion

To provide a measurement of myocardial infarct size in addition to histochemical methods, we assessed CPK release during the initial 24 hours of reperfusion (Fig 5). In both poloxamer 188–treated groups, the areas under the CPK time curves were significantly less than that of the control group (P<.05). There was no significant difference between the two poloxamer 188–treated groups.
Left Ventricular Function

The effect of poloxamer 188 treatment on global and regional left ventricular ejection fractions, assessed by contrast ventriculography, is shown in Fig 6. Baseline ejection fraction did not differ (P=NS) among the groups: 54±1.7% for control, 54±3.0% for 48-hour infusion, and 51±2.0% for 4-hour infusion. As expected, all groups demonstrated a marked reduction in ejection fraction during the coronary artery occlusion (all P<.05 versus baseline; P=NS between groups). The 48-hour infusion of poloxamer 188 resulted in a 38% relative improvement in global left ventricular ejection fraction compared with the control group at 2 hours after reperfusion (47±2.9% versus 34±3.4%, P<.05) and a 30% relative improvement in ejection fraction at 72 hours after reperfusion (43±3.1% versus 33±2.0%, P<.05). The 4-hour poloxamer 188 infusion resulted in an ejection fraction that was intermediate between the control and the 48-hour infusion group at 2 hours after reperfusion (39±3%, P=NS versus control) and at 72 hours after reperfusion (37±1.3%, P=NS versus control). There were no significant differences in ejection fraction between the two poloxamer 188–treated groups at 2 or 72 hours of reperfusion.

Regional wall motion (LAD territory) during coronary occlusion was similarly and severely depressed in

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**Table 4. Myocardial Blood Flow in the Ischemic (Left Anterior Descending) Region and the Nonischemic (Circumflex) Region**

<table>
<thead>
<tr>
<th></th>
<th>Ischemic Region</th>
<th>Nonischemic Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endocardial</td>
<td>Epicardial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endocardial</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.18±0.14</td>
<td>1.08±0.09</td>
</tr>
<tr>
<td>P-188 4-h</td>
<td>1.22±0.14</td>
<td>1.01±0.08</td>
</tr>
<tr>
<td>P-188 48-h</td>
<td>1.17±0.09</td>
<td>1.16±0.13</td>
</tr>
<tr>
<td>Occlusion-60 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.11±0.03*</td>
<td>0.17±0.05*</td>
</tr>
<tr>
<td>P-188 4-h</td>
<td>0.12±0.03*</td>
<td>0.24±0.03*</td>
</tr>
<tr>
<td>P-188 48-h</td>
<td>0.14±0.03*</td>
<td>0.23±0.06*</td>
</tr>
<tr>
<td>Reperfusion-1 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.11±0.12</td>
<td>1.21±0.20</td>
</tr>
<tr>
<td>P-188 4-h</td>
<td>1.23±0.11</td>
<td>0.99±0.12</td>
</tr>
<tr>
<td>P-188 48-h</td>
<td>1.12±0.09</td>
<td>0.87±0.09</td>
</tr>
<tr>
<td>Reperfusion-2 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.20±0.17</td>
<td>0.97±0.11</td>
</tr>
<tr>
<td>P-188 4-h</td>
<td>1.20±0.08</td>
<td>0.99±0.09</td>
</tr>
<tr>
<td>P-188 48-h</td>
<td>0.97±0.12</td>
<td>0.96±0.14</td>
</tr>
</tbody>
</table>

- P-188 4-h indicates poloxamer 4-hour infusion group; P-188 48-h, poloxamer 188 48-hour infusion group. Values are given as mean±SEM.
- *P<.05 vs baseline; no significant differences among groups (by ANOVA).

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![Fig 2](http://circ.ahajournals.org/)

**Fig 2.** Plot of microsphere-determined transmural myocardial blood flow at baseline (before coronary artery occlusion), at 60 minutes of coronary artery occlusion (indicating extent of collateral blood flow), and at 1 and 2 hours of reperfusion. All three groups demonstrated a significant (P<.05) reduction in myocardial blood flow compared with baseline during coronary artery occlusion. There were no significant differences among the treatment groups (by ANOVA). P-188 4-h indicates poloxamer 188 4-hour infusion group; P-188 48-h, poloxamer 188 48-hour infusion group.

![Fig 3](http://circ.ahajournals.org/)

**Fig 3.** Bar graph of effect of poloxamer 188 (P-188) on myocardial infarct size (IN) expressed as a percentage of the area at risk (AR) and as a percentage of the total left ventricle (LV). P-188 4-h indicates poloxamer 188 4-hour infusion group; P-188 48-h, poloxamer 188 48-hour infusion group.
all groups \((P<.01)\). Greater recovery of regional wall motion at both 2 and 72 hours of reperfusion occurred in the poloxamer 188 48-hour group compared with controls \((P<.05)\). In the 4-hour infusion group, regional wall motion at 2 and 72 hours after reperfusion had recovered to an intermediate extent but was not significantly different from the control group.

### In Vitro Neutrophil Chemotaxis and Superoxide Anion Release

The effect of increasing concentrations of poloxamer 188 on neutrophil chemotaxis in vitro is shown in Fig 7.

Poloxamer 188 (0.5 to 2.0 mg/mL) produced a significant reduction in neutrophil chemotaxis \((P<.05\) versus control). Poloxamer 188 plasma concentrations of 1.0 and 2.0 mg/mL (within the range observed during both the 4- and 48-hour infusions) produced neutrophil chemotaxis values of 58% and 52% of control values, respectively.

The effect of increasing concentrations of poloxamer 188 on neutrophil superoxide anion release is shown in Fig 8. Poloxamer 188 concentrations of 1.0 and 3.0 mg/mL were associated with a significant release of superoxide anion, suggesting in vitro neutrophil activation.

### Light Microscopy

The effect of poloxamer 188 on the histopathology of myocardial tissue obtained from the ischemic region 72 hours after reperfusion is shown in Table 5. There were no significant differences among the three groups with respect to hemorrhage or endothelial cell edema. Sim-
Similarly, by 72 hours after reperfusion, only a mild infiltrate of neutrophils and macrophages was seen, and this finding was similar among the groups.

**Discussion**

**Major Study Findings**

The present study demonstrates that a sustained infusion of poloxamer 188 for the initial 48 hours of reperfusion results in a significant reduction in myocardial infarct size and CPK release, along with improved left ventricular function, in this closed-chest canine model of 90 minutes of LAD occlusion and 72 hours of reperfusion. These benefits are independent of differences in collateral blood flow, hemodynamics, and area at risk. The 4-hour infusion of poloxamer 188 resulted in significantly less CPK release and produced nonsignificant trends toward infarct size reduction and improved left ventricular function compared with control. The finding of a better overall effect in dogs receiving the 48-hour infusion of poloxamer 188 supports the existence of a “time window” during which additional myocardial necrosis can occur beyond 4 hours of reperfusion.  

**Prior Studies of Poloxamer 188 in Myocardial Infarction**

Justicz and coworkers studied the effects of poloxamer 188 in open-chest dogs subjected to 90 minutes of LAD occlusion and 24 hours of reperfusion. Although poloxamer 188 (48 mg/kg IV) was infused for only the initial 45 minutes of reperfusion (beginning 15 minutes before reperfusion), they reported a 50% reduction in myocardial infarct size compared with a saline-treated control group (P<.01). Although our study also demonstrated a beneficial effect of poloxamer 188 on infarct size, our results and methods differed in several important ways.

First, despite the fact that we administered higher doses of poloxamer 188 (225 mg/kg during the first hour of infusion followed by 150 mg · kg⁻¹ · h⁻¹ for the next 3 or 47 hours), we found no significant reduction in infarct size by a short-term (4-hour) infusion of poloxamer 188, whereas a sustained (48-hour) infusion significantly reduced infarct size. A possible explanation for our inability to confirm the benefits of a short-term infusion of poloxamer 188, as demonstrated by Justicz and coworkers, may be the longer period of reperfusion used in the present study. The additional 48 hours of reperfusion in our study was important since reperfusion injury can continue to occur beyond 24 hours of reperfusion, and some agents have been shown simply to delay rather than to prevent reperfusion-induced myocardial necrosis. Thus, if Justicz et al had allowed their animals to survive for longer than 24 hours, it is likely that additional reperfusion-induced myocardial necrosis would have occurred, obscuring the benefits of poloxamer 188 apparent at 24 hours.

Second, in the present study we evaluated left ventricular function and demonstrated that the infarct size reduction seen in the 48-hour poloxamer 188 group was associated with a significant improvement in left ventricular ejection fraction and regional wall motion. Third, the potentially confounding effect of variations in collateral blood flow among dogs was accounted for in the present study by the demonstration of a significant difference in the relation between infarct size and collateral blood flow (poloxamer 188 48-hour infusion group versus control, P<.002 by ANCOVA). Fourth, our model more closely simulates the clinical situation because interventions were performed percutaneously, thereby avoiding the potentially confounding effects of thoracotomy. In addition, to avoid external manipulation and ligation of the LAD, an angioplasty balloon was used to internally occlude the LAD, thereby simulating the presence of an occluding atherosclerotic plaque and thrombus.

Other than the study by Justicz et al, the effect of poloxamer 188 in myocardial infarction has not been previously studied. However, poloxamer 188 is a component of the oxygen-carrying perfluorochemical emulsion Fluosol, which has been shown to reduce myocardial infarct size after reperfusion in the dog and rabbit. Fluosol contains 2.7% w/v (27.2 mg/mL) of poloxamer 188, and the doses administered in prior experimental studies have ranged from 15 to 24 mL/kg, administered during the final 15 to 30 minutes of...
coronary occlusion and continuing for the initial 15 to 30 minutes of reperfusion. Although the pharmacokinetics and pharmacological action of poloxamer 188 in Fluosol are unknown, the long plasma half-life of Fluosol may contribute importantly to its beneficial action after myocardial infarction and reperfusion.

Fluosol has also been studied in patients receiving reperfusion therapy for acute myocardial infarction. In a recent randomized pilot study by Forman and coworkers, 26 12 patients with acute anterior myocardial infarction received emergency PTCA alone or PTCA followed immediately by an intracoronary infusion of oxygenated Fluosol (40 mL/min for 30 minutes). Fluosol-treated patients had significantly reduced infarct size, as determined by tomographic thallium scanning, and showed significant improvement in regional left ventricular function. In contrast, a preliminary report 43 demonstrated no significant differences in infarct size or left ventricular function in patients with acute myocardial infarction randomized to receive recombinant tissue-type plasminogen activator and Fluosol IV (15 mL/kg over 1 hour) compared with those receiving recombinant tissue-type plasminogen activator alone. What role, if any, poloxamer 188 played in the outcome of these trials is unknown.

Effects of Poloxamer 188 on Neutrophil Function

A possible mechanism of action is suggested by our in vitro data demonstrating alteration of neutrophil function by poloxamer 188. Poloxamer 188 concentrations of 0.5 to 2.0 mg/mL (within the range of plasma concentrations achieved in this study) produced significant inhibition of neutrophil chemotaxis. These data are in agreement with those of Lane and Lamkin, 22 who showed that inhibition of chemotaxis by Fluosol in washed human neutrophils was due to the Pluronic F-68 (poloxamer 188) component. Fluosol has also been reported to inhibit neutrophil chemotaxis ex vivo. 5

The role of neutrophils in reperfusion injury has been the subject of numerous studies. Several laboratories have reported a dramatic accumulation of neutrophils within the reperfused zone over the initial few hours after reperfusion. 1,12 Inhibition of neutrophil chemotaxis could result in decreased neutrophil infiltration into the postischemic myocardium. Indeed, Justicz et al 23 demonstrated in their canine model of myocardial infarction that treatment with poloxamer 188 was associated with a significant reduction in neutrophil infiltration 24 hours after reperfusion. That the present study failed to demonstrate any difference in neutrophil infiltration into the myocardium among the three groups at 72 hours after reperfusion is probably because the acute inflammatory response had already resolved. Resolution of the acute inflammatory response after 72 hours of reperfusion in the canine model has been noted by other investigators. 9,24,44 We chose a 72-hour duration of reperfusion because of concern that additional reperfusion injury might occur beyond the initial 24 hours.

The mechanism by which poloxamer 188 inhibits neutrophil chemotaxis may result from poloxamer 188–induced neutrophil activation, with subsequent deactivation. This mechanism of chemotactic inhibition has been suggested by prior studies. 24,45,46 We have demonstrated that 1.0 to 2.0 mg/mL poloxamer 188 (within the range of plasma concentrations achieved in the present study) caused significant release of superoxide anion in vitro. These data support the findings of Ingram et al, 45 who reported that poloxamer 188 is the component of Fluosol that is responsible for Fluosol-induced neutrophil activation and release of superoxide anion. Although it may seem paradoxical that an agent that caused neutrophil activation would also be capable of limiting reperfusion injury, our in vitro data support the hypothesis that peripheral activation of neutrophils can lead to deactivation and consequent impairment of neutrophil function (eg, chemotaxis). As they circulate through the reperfused myocardium, deactivated neutrophils should have less capacity to injure vulnerable postischemic myocytes.

Poloxamer 188 might also reduce neutrophil-mediated injury by interfering with neutrophil adhesion. Expression of adhesion proteins on the neutrophil surface may be an important event in the pathogenesis of reperfusion injury. 10,12 Recent studies have demonstrated that antibodies directed against these adhesion molecules can reduce myocardial infarct size in animal models of ischemia and reperfusion. 47 Although the specific effect of poloxamer 188 on adhesion proteins is not known, it has been reported to reduce neutrophil adherence to artificial surfaces such as nylon wool. 22 Inhibition of neutrophil adhesion by poloxamer 188 may be a consequence of its surfactant effect, ie, a nonspecific ability to lower surface tension in aqueous solutions.

Additional Possible Mechanisms for the Beneficial Effect of Poloxamer 188

An additional mechanism by which poloxamer 188 may lessen reperfusion injury is by its beneficial hemorheological properties. Poloxamer 188 lowers blood viscosity 46-50 and decreases red blood cell aggregation 21 in vitro. Despite restoration of blood flow to the infarct-related epicardial coronary artery, microcirculatory flow within the postischemic myocardium may continue to be dysfunctional. This "no-reflow" phenomenon is likely the result of microvascular obstruction due to neutrophil plugging, microthrombi formation, and en-
dothelial cell swelling. Through its hemorheological properties, poloxamer 188 could improve flow through the disordered microcirculation by lessening the natural tendency of cells to stick to one another, thereby acting as a lubricant for bulk flow. Poloxamer 188 has been shown to significantly reduce compromised microcirculatory flow in a canine model of coronary artery thrombotic occlusion and tissue-type plasminogen activator–induced reperfusion. However, in the present study, we did not demonstrate any significant improvement of myocardial blood flow within the reperfused zone at either 1 or 2 hours after reperfusion in any of the three groups.

Clinical Implications

Although definitive clinical evidence supporting the existence of reperfusion injury in humans is lacking, randomized clinical trials are currently in progress with agents targeted to reduce reperfusion injury. Based on the results of the present investigation, a sustained infusion of poloxamer 188 could possibly reduce myocardial infarct size and improve left ventricular function in patients receiving thrombolytic therapy or PTCA for acute myocardial infarction. In addition, experimental data have suggested that poloxamer 188 may enhance the rapidity of thrombolysis with streptokinase or tissue-type plasminogen activator, which would further improve myocardial salvage. Furthermore, poloxamer 188 has been reported to reduce thrombotic occlusion in a swine model of arterial injury, suggesting a potential benefit in reducing recurrent ischemia and reinfarction after successful reperfusion.

Conclusions

The present study has demonstrated that poloxamer 188, a nonionic block copolymer surfactant, caused a significant decrease in reperfusion-induced myocardial necrosis and CPK release while it improved left ventricular function. The mechanism(s) responsible for these benefits may involve alteration of neutrophil function and other beneficial hemorheological effects. The results of this study further demonstrate that a 48-hour infusion of poloxamer 188 was more effective than a 4-hour infusion in reducing myocardial infarct size and improving left ventricular function. This indicates that additional myocardial injury can occur beyond 4 hours of reperfusion. These results hold promise for improving the outcome of patients with acute myocardial infarction.

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Reduction in reperfusion-induced myocardial necrosis in dogs by RheothRx injection (poloxamer 188 N.F.), a hemorheological agent that alters neutrophil function.

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