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Role of Platelet-activating Factor in the Reperfusion Injury of Rabbit Ischemic Heart

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This study shows that the administration of the PAF receptor antagonist SDZ 63-675 (5 mg/kg body weight) before reperfusion significantly reduced the hematologic and hemodynamic alterations, as well as the size of necrotic area in rabbits subjected to 40 minutes of coronary occlusion and reperfusion. Pretreatment with SDZ 63-675 prevented the reduction of platelet counts in the blood obtained from the right ventricle (86.6 ± 2.8% of the control preischemia value) and the transient bradycardia (85.0 ± 2.8%), the systemic hypotension (58.0 ± 2.8%), and the increase in right ventricular pressure (125.0 ± 3.6%) that were evident in the first minutes of reperfusion in untreated control rabbits. Two as well as 24 hours after reperfusion, the infarct size, judged by staining with tetrazolium, was significantly reduced in rabbits treated with SDZ 63-675 (infarct size in control animals, 66.0 ± 2.9% and 63.46 ± 2.09% of the risk region at 2 or 24 hours, respectively, compared with 38.9 ± 5.2% and 37.11 ± 2.44% of the risk region at 2 and 24 hours in rabbits treated with SDZ 63-675). This result was confirmed by histologic examination of cardiac tissue 24 hours after reperfusion. In addition, SDZ 63-675 markedly reduced the accumulation of ^{111}In-oxine–labeled platelets that occurs 15 minutes after reperfusion in the central ischemic area of the heart and in the lungs. These results suggest that PAF plays a role in the evolution of myocardial injury observed during reperfusion. (Am J Pathol 1990, 137:71–83)

Several experimental and clinical studies have suggested that timely reperfusion of ischemic myocardium may reduce the extension of necrosis after coronary artery occlusion. However, from these studies also emerged the fact that reperfusion, while terminating ischemia, may produce a specific reperfusion injury. The cellular damage produced by reperfusion is additive to that produced by ischemia alone, and may jeopardize the beneficial effects of restoring the normal circulation in the coronary artery.[1–14] Several mechanisms have been implicated in reperfusion injury: the generation of oxygen-free radicals at the time of reperfusion,[15–22] the release of vasoactive mediators, such as thromboxane A_2[23–27] and leukotrienes,[28] and the enhanced degradation of membrane phospholipids and turnover of phosphoinositides.[29–31]

We have recently demonstrated that platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) is released in the coronary effluent early after reperfusion in the ischemic isolated rabbit heart.[32] The amount of PAF released in this experimental condition is sufficient to induce an intracoronary activation of platelets with release of secondary mediators.[32] This observation suggests the possibility that PAF may contribute to the cardiac dysfunction observed after reperfusion. In fact, reperfusion injury has been at least in part related to the formation of intracoronary platelet thrombi and especially recruitment of leukocytes, that interacting with endothelium may promote increase in vascular resistances, reduce collateral flow, favor vasospasm, and mediate the enhancement in vascular permeability.[33–37]

The wide range of biologic activities of PAF, and especially its ability to induce aggregation and degranulation of platelets[38] and to promote adherence to endothelium, chemotaxis, and granule secretion of polymorphonuclear

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neutrophils[39,40] and monocytes,[41] makes PAF a potential candidate in mediating several of the phenomena observed during the reperfusion injury. In addition, the negative inotropic effect of PAF and its arrhythmogenic and vasospastic properties[42–48] can contribute to the myocardial dysfunction defined as 'myocardial stunning,' observed during reperfusion.

The aim of the present study is to evaluate the potential role of PAF in the reperfusion injury by administering a specific PAF-receptor antagonist (SDZ 63-675)[49] before reperfusion in a rabbit model of experimentally induced myocardial infarction.

Materials and Methods

Animals

Sixty-five New Zealand white rabbits of both sexes weighing 2.4–3.2 kg and fed with a standard diet were used. The animals were anesthetized with ketamine (7 mg/kg intravenously, Ketalar, Parke-Davis, Milano, Italy) and maintained under continuous infusion of ketamine (0.8 mg/kg/minute). The depth of anesthesia was ascertained by the lack of the corneal reflex.

Surgical Procedure

Rabbits were intubated through a tracheotomy and ventilated with room air (Small Animal Respirator, Harvard Apparatus, Edenbridge, England). Sterile, saline-filled, heparinized catheters (3 French, Biotrol Pharma, Paris, France) were inserted into ascending aorta via the left common carotid artery and into the right ventricle via the jugular vein for pressure recording and blood sampling. A left thoracotomy was performed at the fifth intercostal space of each rabbit, incising the pericardium and temporarily occluding the large marginal branch of the circumflex coronary artery about 3 mm from its origin by a surgical suture snare. In the rabbit, the anterior descending coronary artery is a small vessel, the anterior and lateral left ventricular wall is consistently supplied by a large marginal branch of the circumflex artery.[50] Coronary artery occlusion (CAO) was maintained for 40 minutes, at which time the ligature was released and reperfusion continued for 2 or 24 hours. The chest cavity was closed after reperfusion and the animals were removed from the respirator.

Experimental Design

Rabbits were divided into seven experimental groups.

Group 1: Ten rabbits received, 5 minutes before reperfusion, via a peripheral vein, a 2-ml bolus of standard solution (glucose 5% in sterile water) (control rabbits).

Group 2: Ten rabbits received, 5 minutes before reperfusion, a 2-ml bolus of standard solution containing 5 mg/kg SDZ 63-675 (Sandoz, East Hanover, NJ), a PAF-receptor antagonist.[49]

The rabbits of groups 1 and 2 were killed 2 hours after the beginning of reperfusion. Mean arterial pressure (MAP) and right ventricular pressure (RVP) were measured by means of Harvard 377 pressure transducers, recorded on a magnetic tape by a 3964 A Hewlett-Packard recorder (Palo Alto, CA), visualized on a Tektronix 5103 N oscilloscope (Beaverton, OR) and reproduced by a Hewlett-Packard 7015 B X-Y recorder for data analysis. In five rabbits of group 1 and five rabbits of group 2, venous and arterial blood samples were obtained, via catheters placed in the right ventricle and ascending aorta, before CAO, 30 and 40 minutes after occlusion, and at 1, 3, 15, 30, 60, and 120 minutes after reperfusion for determination of the following parameters: hematocrit (Hct), hemoglobin concentration (Hb), number of platelets (PTLS), and leukocyte count (WBC). Blood samples (500 μl) were placed in tubes containing ethylenediaminetetra-acetic acid (EDTA). Hematocrit, Hb, PTLS, and WBC counts were determined by a Coulter counter (Electronics Inc, Hialeah, FL). The values were expressed as percent variation from baseline, assumed as 100%. In five rabbits of group 1 and five rabbits of group 2, regional myocardial blood flow (RMBF) was measured 30 minutes after occlusion and 2 hours after reperfusion (see below).

Group 3: Fifteen control rabbits received standard solution as in group 1, and were killed 24 hours after the beginning of reperfusion.

Group 4: Fifteen rabbits received standard solution containing SDZ 63-675 as in group 2, and were killed 24 hours after the beginning of reperfusion.

In 10 rabbits of group 3 and 10 rabbits of group 4, RMBF was measured 30 minutes after CAO and 15 minutes after reperfusion. To evaluate the biodistribution of platelets in nonischemic as well as control and SDZ 63-675–pretreated rabbits, the following experiments were done:

Group 5: To evaluate the biodistribution of radiolabeled platelets in nonischemic animals, five control sham-operated (thoracotomy without CAO maintained for 55 minutes) rabbits received an intravenous injection of 1 ml ^{111}In-oxine–labeled platelets[51] ($4.8 \pm 0.4 \times 10^9$/ml).

Group 6: Five rabbits received an intravenous injection of 2 ml standard solution 5 minutes before the beginning of reperfusion and 1 ml of ^{111}In-oxine–labeled platelets ($4.6 \pm 0.5 \times 10^9$/ml) at the time of reperfusion.

Group 7: Five rabbits received an intravenous injection of 2 ml standard solution containing 5 mg/kg SDZ 63-675 5 minutes before the beginning of reperfusion, and 1 ml of ^{111}In-oxine–labeled platelets ($4.7 \pm 0.3 \times 10^9$/ml) pre-

treated with 3 micromolar (μmol/l) SDZ 63-675 at the time of reperfusion.

As described in the section Biodistribution of Radiolabeled Platelets, rabbits were killed after 15 minutes, and accumulation of radioactive platelets was evaluated in the following organs: heart, lungs, spleen, left kidney, and liver.

During the experiments, the electrocardiogram (ECG) (lead II) was recorded by means of silver electrodes, amplified with a Tektronix AM 502 differential amplifier and recorded on magnetic tape. The following ECG parameters were considered: heart rate (HR) and ST segment alteration.

Regional Myocardial Blood Flow (RMBF) Measurement

Regional myocardial blood flow was determined by the radioactive microspheres technique.[52-55] Chromium 51 and Cerium 141 labeled microspheres (Du Pont de Nemours GmbH, Dreieich, West Germany) measuring 16.5 ± 0.1 μ were suspended in 0.9% saline solution plus 0.01% Tween 80 (ICI Americas, Wilmington, DE) to minimize clumping. In each experiment, 250 μl of the stock solution, containing about 300,000 microspheres, was vigorously agitated in a plastic syringe, to ensure good dispersion, and injected into the left atrium. A sterile, heparinized, and saline-filled polyethylene cannula was inserted into the left femoral artery, and it was connected to a Harvard electric pump for blood withdrawal. The withdrawal of blood from the left femoral artery began 10 seconds before the injection of the microspheres at a steady rate of 1 ml/minute; the microspheres were injected within 20 seconds, and the blood withdrawal lasted for a further 30 seconds. The first injection of microsphere was given 30 minutes after coronary occlusion. The second injection of microspheres was made 2 hours after reperfusion in the rabbits of groups 1 and 2 and 15 minutes after reperfusion in the rabbits of groups 3 and 4. Animals were killed immediately after the second injection of micropheres by an overdose of ketamine and KCl (0.2 molar [M]).

Samples for determination of RMBF were obtained from endocardial and epicardial sections in the nonischemic area (delineated by Monastryl blue dye), in the center of ischemic zones, unstained by triphenyltetrazolium chloride (TTC; central zone = 55% of the vascular bed at risk), and in the lateral region at risk. The lateral border zones were excluded from analysis to avoid misinterpretation of measurement from samples that might have contained nonischemic as well as ischemic myocardium. In rabbits killed 24 hours after the beginning of reperfusion, tissue sampling for the analysis of RMBF and histology was performed according to the method described by Reimer et al.[56] Briefly, alternative sections were used for the evaluation of RMBF and histologic extension of infarct size. Myocardial sections and arterial blood reference samples were counted for radioactivity by a multichannel gamma scintillation counter (Hewlett-Packard model 5400) and myocardial blood flow was determined (in milliliters per minute per gram of tissue) by methods previously described.[54]

Analysis of the Region at Risk and of Infarct Size

The size of anatomic risk region, or occluded vascular bed, was measured by *ex vivo* perfusion technique.[57] The aorta was cannulated above the coronary ostia. The coronary vasculature first was perfused with 30 ml warm (37°C) saline. The coronary artery then was reoccluded at the same site and the heart was perfused again with 40 ml of a Monastryl blue dye (0.5%) suspended in 6% dextran 70 in normal saline.[58] Both solutions were perfused through the same cannula at a constant pressure of 75 mm Hg. The excised heart of each rabbit was weighed and the left ventricle was sliced in six slices parallel to the atrioventricular groove. The total cross-sectional area of the slices and of the area at risk (that unstained by Monastryl blue dye) were determined by planimetry. The slices then were incubated in a 1% TTC solution in phosphate buffer for 20 minutes at 37 °C, pH 7.4.[19] The region of infarcted myocardium in the area of risk was demarcated by the absence of TTC staining and determined by planimetry. Areas of the left ventricular rings, risk regions, and infarcts were calculated by multiplying the appropriate area ratios by the weight of each ring and summing the values for whole heart. The ratio of the area of necrosis to the area at risk and the ratio of area of necrosis to the area of the left ventricle were calculated. To check the accuracy of estimation of the infarct size by gross pathology, the left ventricular slices were fixed in 10% neutral buffered formalin, conventionally processed, and embedded in paraffin. Histologic sections (6 μ thin) of the same tissue faces viewed by TTC staining were stained by hematoxylin and eosin (H&E). Histologic sections likewise were magnified to 12×, and the necrotic region and total left ventricle were planimetrically assessed. The histologic definition of necrotic areas was based on the classic criteria of coagulative necrosis (increased hypereosinophilia, granularity of cytoplasm, nuclear pyknosis, and karyolysis).[59,60] The loss of cytoplasmic cross-striations, interstitial edema, hemorrhage, and inflammatory infiltrates were also present, but they were not considered diagnostic for determination of necrotic areas in the heart.[59,60]

Biodistribution of Radiolabeled Platelets

Washed rabbit platelets were prepared as described by Ardlie et al.[61] Blood was drawn into 50-ml plastic tubes containing 1×10^{-3} M (final concentration) EDTA, pH 7.2, and centrifuged at room temperature at 375*g* for 20 minutes.[32] The platelet-rich plasma (PRP) was removed and centrifuged 2 times at 1400*g* for 15 minutes with intermediate washing in TRIS-buffered Tyrode-gelatin (TTG) solution without Ca^{++} and Mg^{++}, pH 6.5, that consisted of Tyrode's solution (composition, in mmol/l (millimolar): 137 NaCl, 4.0 KCl, 5.5 glucose, 10 $NaHCO_3$) containing 1 mmol/l TRIS(hydroxymethyl)aminomethane (TRIS), and 0.25% gelatin (Difco Laboratories, Detroit, MI). Platelets ($4.7 \pm 0.7 \times 10^9$/ml) then were gently resuspended in 10 ml TTG. Approximately 35 μCi of ^{111}In-oxine (Amersham International plc, Buckinghamshire, UK) were added dropwise to 10 ml of the platelet suspension and allowed to incubate at room temperature for 10 minutes. After incubation, the platelet suspension was centrifuged at 1400g for 10 minutes and the platelet pellet was resuspended in 2 ml sterile saline. Labeling efficacy, defined as (^{111}In bound to platelets / ^{111}In bound to platelets + unbound ^{111}In activity) $\times$ 100, was 96.1 $\pm$ 0.7% (range, 93% to 98%). The function of ^{111}In-labeled platelets was assessed by an Aggregometer Elvi 480 (Logos I, Milano, Italy), using synthetic 5×10^{-11} - 1×10^{-9} M PAF (Bachem Feinchemikalien, Bubendorf, Switzerland) as aggregating agent. ^{111}In-labeled platelets were infused within 1 minute in the jugular vein at the time of reperfusion. After 15 minutes of reperfusion, rabbits were killed and the size of anatomic risk region was determined after *ex vivo* perfusion as described in the section Analysis of the Region at Risk and of Infarct Size. Samples for quantitation of ^{111}In-labeled platelets accumulated in myocardial tissue were obtained for transmural sections of nonischemic area and the center of ischemic zones (55% of the vascular bed at risk). Tissue sections were weighed and placed in a gamma spectrometer (Hewlett-Packard model 5400) to determine the amount of ^{111}In radioactivity present in each piece of tissue. The accumulation of ^{111}In-labeled platelets is expressed as the increase in ^{111}In radioactivity in the central ischemic transmural sections of myocardium over ^{111}In radioactivity in transmural sections of normal myocardium, according to a modification of the method of Golino et al.[57] The radioactivity counts of the central ischemic and nonischemic myocardium were divided by the tissue wet weight to obtain counts per minutes per gram of tissue. By dividing the activity of the central area at risk (cpm/g) by the activity of nonischemic tissue (cpm/g), the increase in ^{111}In radioactivity in the central ischemic zone was calculated, yielding the platelet accumulation ratio. Multiple samples of lungs, spleen, liver, and left kidney were obtained. The radioactivity was expressed as percentage of the injected dose per organ, using the following assumptions: skeletal muscle mass, 43% of the total body weight (TBW); skeletal mass, 10% TBW, and bone marrow mass, 2.2% TBW, as described by Hill-Zobel et al.[62]

Statistical Analysis

Data are presented as the mean $\pm$ standard error (SE). Student's *t*-test for paired data was used for statistical comparison between groups 1 through 4. Because of the major importance of area at risk and collateral flow in determining infarct size, these parameters were incorporated into the statistical analysis of infarct size. Infarct size in control and SDZ 63-675 treated rabbits (as percent of area at risk) was compared by the analysis of covariance using collateral blood flow as the covariate, as described by Reimer et al.[56] The correlation between infarct size (percentage of left ventricle [LV]) and area at risk (percentage of LV) in control and SDZ 63-675–treated rabbits, and gross TTC and microscopic measurements of LV infarct size was assessed by linear regression analysis. Statistical analysis between groups 5 through 7 was performed using one-way analysis of variance (ANOVA). If a significant F resulted from the analysis of variance ($P < 0.05$), the Newman-Keuls' multiple range test was applied to determine where differences were located among the groups.

Results

Circulating Platelets and Leukocyte Counts

The number of platelets in venous and arterial blood samples obtained before and 30 and 40 minutes after CAO was not significantly different in rabbits of group 1 *versus* rabbits of group 2 (Figure 1). A reduction in the number of platelets was observed in venous samples from rabbits of group 1 at 1, 3, and 15 minutes of reperfusion. In arterial blood samples, a significant decrease in platelet counts was measured at only 3 minutes of reperfusion. Pretreatment with the PAF receptor antagonist SDZ 63-675 (rabbits of group 2) completely abrogated the reduction in the number of platelets during reperfusion in both venous and arterial blood samples. In contrast, the variations of leukocyte counts observed during coronary occlusion and reperfusion were not significantly different between rabbits of groups 1 and 2 (Figure 1). No significant variation of Hct and Hb were observed in groups 1 and 2 through all the experimental duration (data not shown).

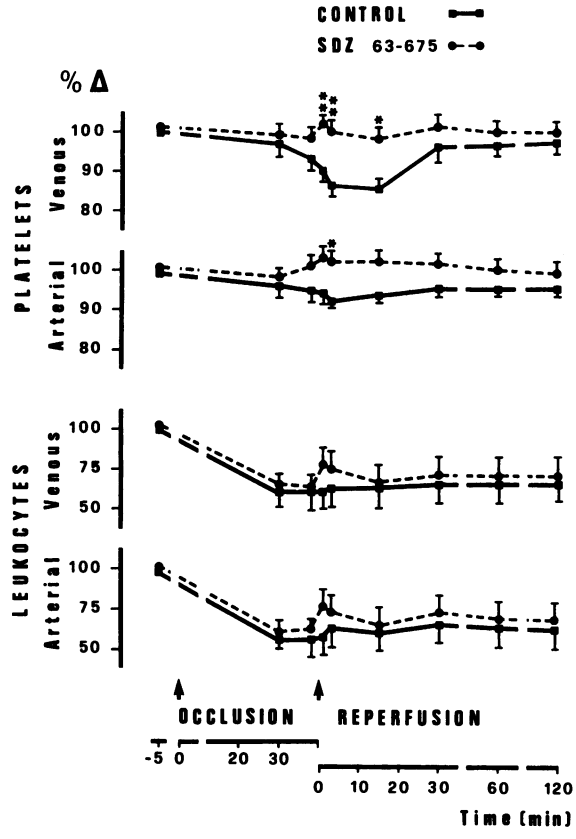

Figure 1. *Hematologic alterations during ischemia and reperfusion in control (group 1, n = 5) and SDZ 63-675-treated rabbits (group 2, n = 5). Data are expressed as the mean ± SE percentage change from the preischemia value (*, P = 0.05; **, P = 0.01 by Student's t-test). Baseline values for group 1 rabbits were: platelets = 490 ± 43 × 10^3/μl, leukocytes = 5.03 ± 0.68 × 10^3/μl (arterial blood samples); platelets = 474 ± 46 × 10^3/μl, leukocytes = 5.11 ± 0.51 × 10^3/μl (venous blood samples). Baseline values for group 2 rabbits were: platelets = 523 ± 54 × 10^3/μl, leukocytes = 5.01 ± 0.50 × 10^3/μl (arterial blood samples); platelets = 507 ± 48 × 10^3/μl, leukocytes = 4.89 ± 0.61 × 10^3/μl (venous blood samples).*

Hemodynamic Studies

Preocclusion heart rate (HR), mean arterial pressure (MAP), and right ventricular pressure (RVP) were similar in rabbits of group 1 and 2. After coronary occlusion, HR did not change appreciably, while MAP decreased, RVP increased slightly (Figure 2), and a marked ST segment elevation occurred. In the rabbits of group 1, a significant decrease in MAP, which was present in the first 10 to 15 minutes of reperfusion, was accompanied by a sustained bradycardia and an increase in RVP that persisted for up to 30 minutes of reperfusion. In rabbits of group 2, pretreated with SDZ 63-675, all the hemodynamic effects observed during reperfusion were significantly reduced in entity, and bradycardia was completely abrogated.

Regional Myocardial Blood Flow

Rabbits of groups 1 and 2 were equally ischemic at 30 minutes after occlusion (Figure 3). In fact, transmural mean RMBF in the central ischemic zone was 0.30 ± 0.06 *versus* 0.37 ± 0.11 ml/minute/g in control and treated animals, respectively (*P* = not significant [ns]). SDZ 63-675 given at the time of reperfusion did not increase blood flow to the previously ischemic myocardium at 2 hours after reperfusion (Figure 3). Transmural blood flow in the nonischemic zone did not differ between the two groups during occlusion (3.15 ± 0.64 *vs.* 3.32 ± 0.47) and after reperfusion (2.85 ± 0.43 *vs.* 2.48 ± 0.43). As reported by other investigators, RMBF values in rabbits are considerably higher than those reported in other species.[52,63] Hearts obtained from rabbits of group 3 and 4 did not exhibit at 30 minutes after occlusion significant difference in RMBF in the central ischemic zone (0.29 ± 0.06 *vs.* 0.33 ± 0.07), or in the nonischemic zone (3.06 ± 0.13 *vs.* 3.11 ± 0.18).

Similarly, no variation was observed in endocardial and epicardial regions (data not shown). Rabbits treated with SDZ 63-675 (group 4) did not exhibit a significant differ-

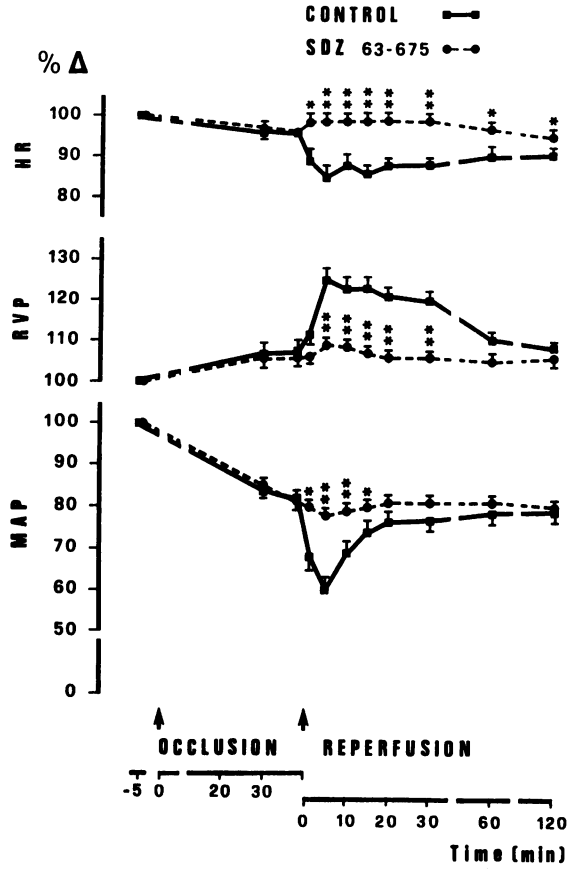

Figure 2. *Circulatory alterations during ischemia and reperfusion in control (group 1, n = 10) and SDZ 63-675-treated rabbits (group 2, n = 10). HR = heart rate; MAP = mean arterial pressure; RVP = right ventricular pressure. Data are expressed as the mean ± SE percentage change from the preischemia value (*, P = 0.05; **, P = 0.01 by Student's t-test). Baseline values for group 1 rabbits were: HR = 232.4 ± 6.6 beats/minute; MAP = 88.3 ± 7.2 mm Hg; RVP = 18.3 ± 2.6 mm Hg; baseline values for group 2 rabbits were: HR = 225.4 ± 5.9 beats/minute; MAP = 84.6 ± 5.0 mm Hg; RVP = 18.9 ± 3.1 mm Hg.*

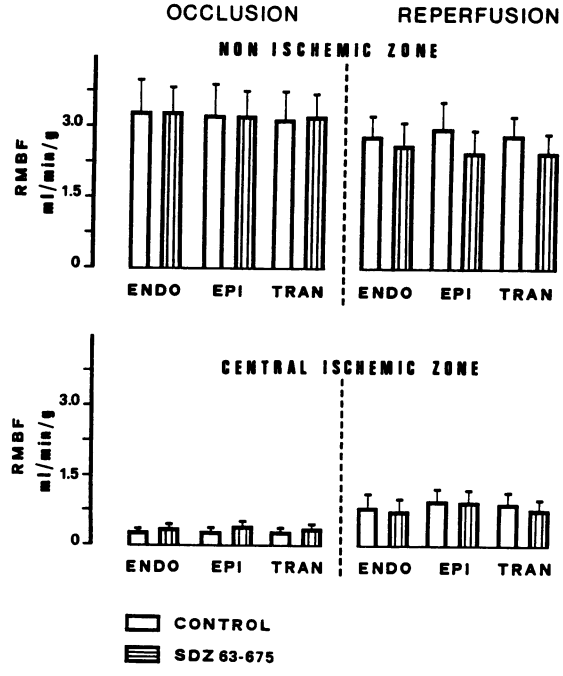

Figure 3. *Regional myocardial blood flow (RMBF, expressed as ml/min/g cardiac tissue) in the nonischemic zone and in the central ischemic zone measured 30 minutes after occlusion and at 2 hours after reperfusion in control (group 1, n = 5) and SDZ 63-675 (group 2, n = 5)-treated rabbits. Data are expressed as mean ± SE. ENDO = endocardium; EPI = epicardium; TRAN = transmural.*

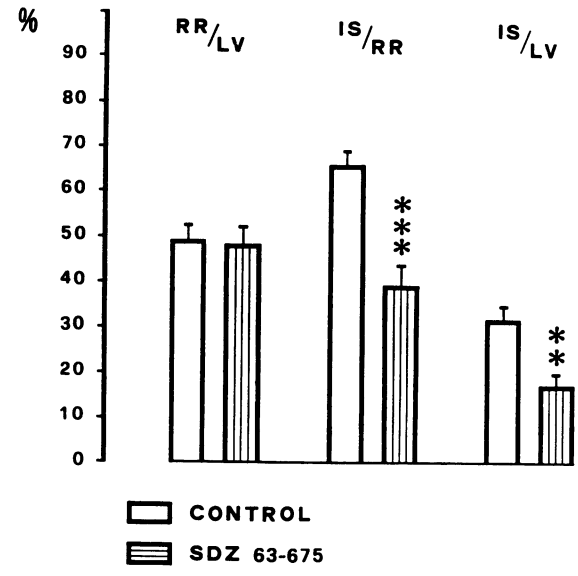

Figure 4. *Effect of SDZ 63-675 on infarct size (IS) in rabbits subjected to 40 minutes of coronary artery occlusion and 2 hours of reperfusion. Data are expressed as the mean ± SE percentage of the region at risk (RR), or total left ventricle (LV) (**, P < 0.01; ***, P < 0.001 by Student's t-test).*

ence in RMBF 15 minutes after reperfusion with respect to control rabbits (group 3) in the central ischemic zone (3.42 ± 0.46 *vs.* 3.05 ± 0.32) or in the nonischemic zone (2.75 ± 0.13 *vs.* 2.80 ± 0.17). This absence of reduction in RMBF at 15 minutes of reperfusion in the central ischemic zone was due to the hyperemia occurring in the early phases of reperfusion. In the rabbit, the reduction in RMBF in the central ischemic zone became detectable after 2 hours of reperfusion probably is due to the histologic progression of myocardial injury.[64]

Area at Risk and Infarct Size

The size of anatomic area at risk in rabbits of group 1 and 2 was superimposable ($P = 0.20$). Occlusion of the coronary artery resulted in an occluded bed size of 49.4 ± 3.6% of the left ventricle in control rabbits and of 48.5 ± 4.0% in treated rabbits (Figure 4).

In rabbits of group 1, the infarct size, calculated as a percentage of the left ventricle, was 32.8 ± 3.0%. In contrast, in SDZ 63-675–treated rabbits, infarct size was only 17.8 ± 3.0% ($P < 0.01$). Moreover, infarct size, normalized as a percentage of the area at risk, was larger in control rabbits (66.4 ± 2.9%), when compared with rabbits pretreated with SDZ 63-675 (38.9 ± 5.2%) ($P < 0.001$; Figure 4). Because collateral blood flow is considered an important factor affecting myocardial infarct size,[56] the data also are plotted as a function of collateral blood flow (Figure 5). Platelet-activating factor receptor antagonist reduced infarct size/risk region for any given blood flow. However, Figure 5 shows no statistical correlation between collateral blood flow and IS/RR. This result, different from that reported in dogs,[56] depends on the fact that, in rabbits, collateral blood flow was shown to be uniformly low and insufficient to modify the perfusion of the area at risk.[65] Rather than collateral blood flow, the exten-

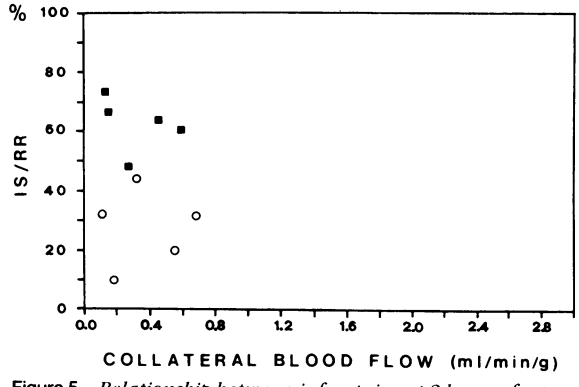

Figure 5. *Relationship between infarct size at 2 hours of reperfusion normalized to region at risk (IS/RR) and central transmural collateral blood flow at 30 minutes after occlusion for group 1 (■, n = 5) and group 2 (○, n = 5) rabbits. Each point represents an individual rabbit. IS/RR compared between group 1 and group 2 rabbits by analysis of covariance using collateral blood flow as covariate: P < 0.01. Infarct size was proportional to risk region in both group 1 (r = 0.96) and group 2 (r = 0.84).*

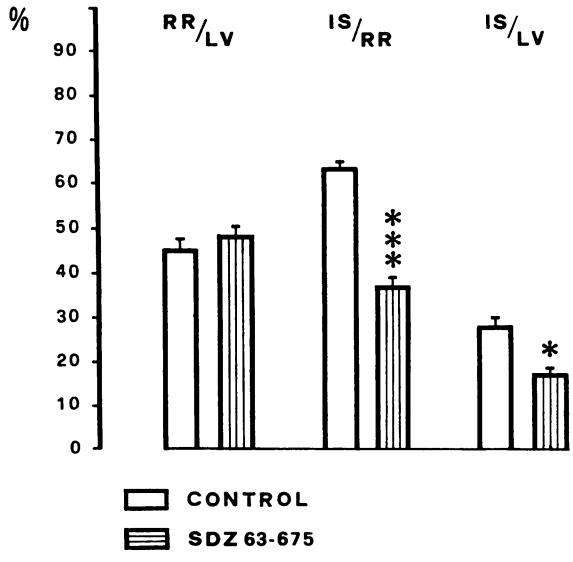

Figure 6. *Effect of SDZ 63-675 on infarct size (IS) in rabbits subjected to 40 minutes' coronary artery occlusion followed by 24 hours of reperfusion. Data are expressed as the mean ± SE percentage of the region at risk (RR), or total left ventricle (LV) (*, P < 0.05; ***, P < 0.001 by Student's t-test).*

sion of the infarct size depends in rabbits on the extension of the area at risk.

After 24 hours of reperfusion, groups 3 and 4 were similar with respect to size of area at risk (45.2 ± 2.9% *vs.* 47.9 ± 2.8%). In contrast, infarct size, detected by TTC, expressed both as a percentage of the risk region and as a percentage of the left ventricular mass, was significantly reduced in the SDZ 63-675–treated group, as compared with the control animals (Figure 6). The relationship between infarct size normalized to the area at risk and transmural mean RMBF in the central ischemic zone at 30 minutes after occlusion is illustrated in Figure 7. This result was confirmed by histologic examination of cardiac tissue (Figure 8). Histologically, the mean infarct size, normalized to risk region, was 66.2 ± 3.7% in control rabbits (group 3) and 37.1 ± 3.4% in rabbits treated with SDZ 63-675 (group 4) ($P < 0.001$); infarct size, calculated as a percentage of the left ventricle, was 30.1 ± 2.1% and 18.6 ± 2.1% in rabbits of groups 3 and 4, respectively ($P < 0.05$). The comparison of planimetry measurements of 90 gross slices and microscopic giant histologic sections is shown in Figure 9. There was close correspondence between gross and histologic measurements of areas of necrosis in each slice ($r = 0.931$).

Organ Distribution of ^{111}In-labeled Platelets

Fifteen minutes after reperfusion, the ratio of myocardial platelet accumulation in control (group 5) and SDZ 63-675 treated (group 6) rabbits was 5.63 ± 0.79 and 3.41 ± 0.93, respectively (Figure 10). SDZ 63-675 significantly reduced the ratio of platelet accumulation ($P < 0.05$). As shown in Table 1, SDZ 63-675 reduced also the accumulation of ^{111}In-oxine–labeled platelets in heart and lungs, but not in other organs.

Discussion

The present study suggests the role of PAF in tissue injury of ischemic reperfused rabbit heart. The PAF receptor antagonist SDZ 63-675 given before reperfusion significantly reduced the infarction area as judged by histochemical and histologic examination. Two, as well as 24, hours after the beginning of reperfusion, the necrotic area judged by staining with tetrazolium was markedly reduced in rabbits treated with SDZ 63-675 as compared with untreated controls. These results were confirmed by histologic examination of cardiac tissue 24 hours after reperfusion. At this time, the extension of the infarcted area was reduced to about 58% in rabbits treated with SDZ 63-675. No significant differences in hemoglobin concentration, extension of hypoperfused area, or collateral coronary flow during ischemia were observed in SDZ 63-675–treated and untreated rabbits. However, rabbits treated with SDZ 63-675 lacked the early reduction of platelet counts obtained in the blood from the right ventricle and the early, transient hypotension that were present in the untreated rabbits. These results suggest that PAF plays a role in the evolution of myocardial injury observed during reperfusion of ischemic area. Platelet-activating factor in fact is present in the perfusate of ischemic reperfused isolated rabbit heart.[32] Because PAF is detectable only in the early phase of reperfusion, it has been suggested that

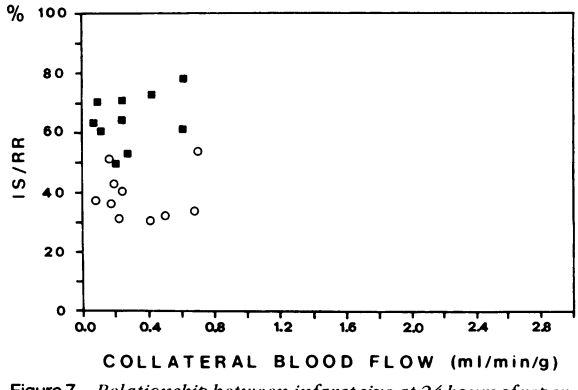

Figure 7. *Relationship between infarct size at 24 hours of reperfusion normalized to region at risk (IS/RR) and central transmural collateral blood flow at 30 minutes after occlusion for group 3 (■, n = 10) and group 4 (○, n = 10) rabbits. Each point represents an individual rabbit. IS/RR compared between group 3 and group 4 rabbits by analysis of covariance using collateral blood flow as covariate: P < 0.001. Infarct size was proportional to risk region in both group 3 (r = 0.93) and group 4 (r = 0.82).*

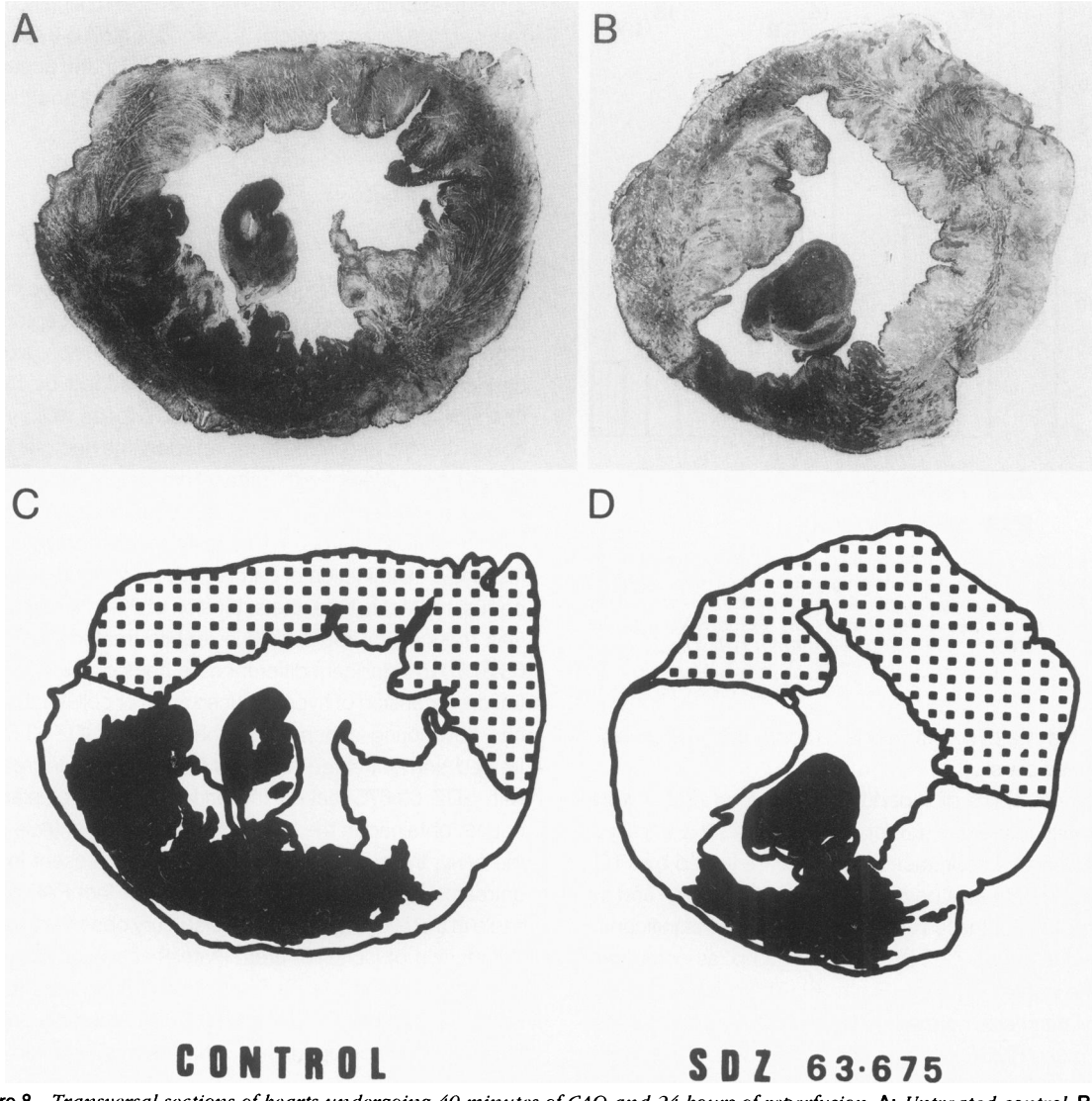

Figure 8. *Transversal sections of hearts undergoing 40 minutes of CAO and 24 hours of reperfusion.* **A:** *Untreated control;* **B:** *SDZ 63-675–treated rabbit (H&E, original magnification × 12).* **C and D:** *Schematic representation of the same section shown in A and B, respectively (dotted area = nonischemic area; dark area = necrotic area; white area = risk region, determined as described in Materials and Methods). The treatment with SDZ 63-675 markedly reduced the infarct size (necrotic area) in spite of a superimposable hypoperfused area (risk region).*

the release of this mediator takes place during this phase rather than during the ischemia. It has been shown recently that endothelial cells release PAF after stimulation with hydrogen peroxide.[66] Oxygen radicals have been documented to be generated during reperfusion injury of the heart.[67] It therefore is possible that PAF synthesis may be triggered by this mechanism. Besides endothelial cells,[68,69] other cells in the heart, such as myocardiocytes,[70] platelets,[71] and inflammatory cells[72–74] recruited during ischemia, may produce PAF. However, we can not exclude that PAF may be synthesized and accumulated during the ischemic phases, to be eventually released in the blood when circulation is restored. It therefore is possible that a local concentration of PAF may be significantly higher than that detectable in the coronary effluent. The experiments in the isolated ischemic and reperfused rabbit heart indicate that in this animal species platelets are required for mechanical, electrical, and vascular alterations induced by PAF.[32]

Platelet-activating factor released during reperfusion may influence an early intracardiac activation of platelets, as suggested by the early transient thrombocytopenia associated with an accumulation of ^{111}In-labeled platelets in the central ischemic zone and by the protective effect of a PAF receptor antagonist. Platelet-activating factor, in addition, can recruit inflammatory cells in the site of necrosis,[39–41] may increase vascular permeability,[75] and may affect vasodynamic phenomena,[42,44,45,47,48] thus propa-

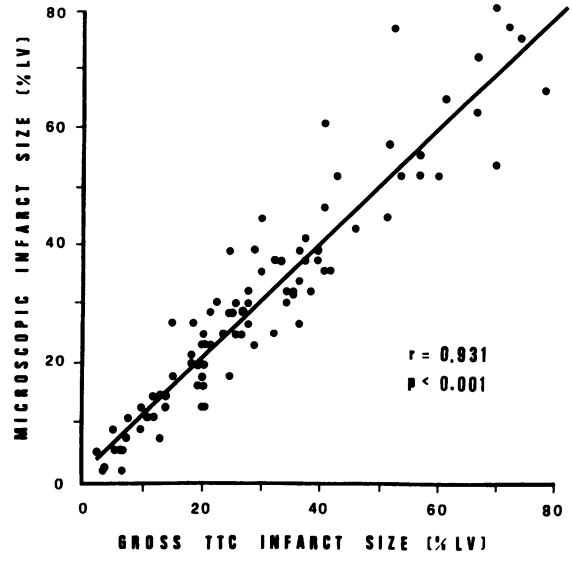

Figure 9. *Linear regression analysis comparing gross TTC and microscopic measurements of LV infarct size.*

gating the injury caused by ischemia. Mickelson et al[64] have recently shown that PAF administration after coronary occlusion and reperfusion of rabbit heart determines increase in coronary resistances and decrease in left ventricular developed pressure. These phenomena have been related to the release of cyclooxygenase and lipoxygenase products by PAF-stimulated platelets and leukocytes accumulated in the infarcted area.[76]

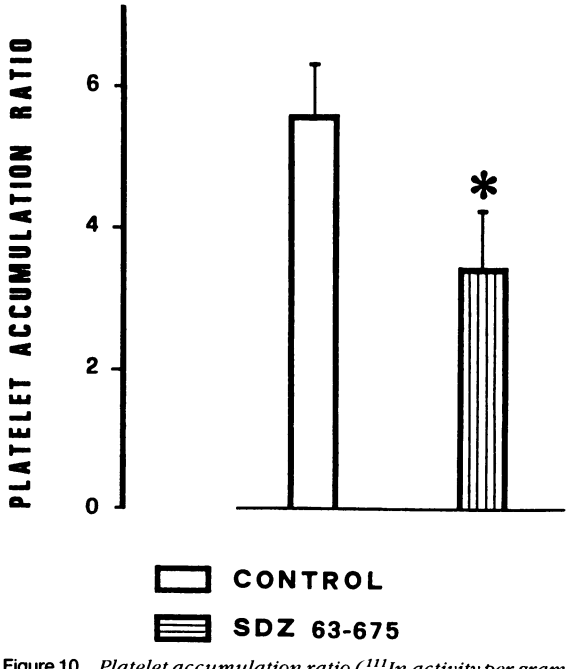

Figure 10. *Platelet accumulation ratio (^{111}In activity per gram of tissue in the central ischemic myocardium/^{111}In activity per gram of tissue in nonischemic myocardium) in group 5 and group 6 rabbits after 40 minutes of occlusion and 15 minutes of reperfusion. Vertical bars represent ± SE.* *, $P < 0.05$.

Table 1. *Organ Distribution of ^{111}In-labeled Platelets in Sham-operated (Group 5), in Control (Group 6), and SDZ 63-675–treated (Group 7) Rabbits Subjected to Coronary Artery Occlusion and Reperfusion*

	Group 5 rabbits (n = 5)	Group 6 rabbits (n = 5)	Group 7 rabbits (n = 5)
Heart	0.15 ± 0.03	0.28 ± 0.06*	0.16 ± 0.06‡
Lung	1.46 ± 0.21	4.96 ± 0.69†	1.96 ± 0.35‖
Spleen	3.74 ± 0.77	2.20 ± 0.62*	2.12 ± 0.35*
Kidney, left	0.35 ± 0.03	0.51 ± 0.08*	0.40 ± 0.08
Liver	20.40 ± 3.37	19.15 ± 4.66	16.07 ± 2.43

Values are expressed as the mean ± SE% of injected dose.
Statistical analysis was performed comparing group 5 with group 6 and group 7 (*$P < 0.05$; †$P < 0.01$) or group 6 with group 7 (‡$P < 0.05$; ‖$P < 0.01$).

Several experimental models support the role of leukocytes in the reperfusion injury of the heart. During ischemia, margination, adhesion, and diapedesis of neutrophils were observed in the coronary vessels of ischemic area. The formation of intravascular aggregates of neutrophils has been proposed as a mechanism of no-reflow phenomenon in the ischemic-reperfused heart.[33–37] Platelet-activating factor therefore may be involved in the recruitment and intravascular aggregation of neutrophils, as well as in their activation with generation of oxygen-free radicals, release of proteolytic enzymes,[34–37] and activation of arachidonic acid-dependent pathways.[76] In addition, PAF, which is 100 times more potent than histamine in increasing vascular permeability,[75] may propagate ischemic injury as consequence of diffuse perivascular and interstitial oedema. Stahl et al[77] demonstrated that CV 3988 prevents the increased vascular permeability observed in the ischemic reperfused rat heart. In addition, PAF has direct effects on coronary flow, myocardial contractility, and cardiac rhythm in several species, including man.[42–48] In the present model, the direct effect of PAF in coronary blood flow and myocardial contractility is probably not involved, because the isolated perfused rabbit heart is rather insensitive to this mediator.[32] However, the relative low percentage of platelets accumulated in ischemic and reperfused heart with respect to the amount of platelets trapped in the peripheral circulation suggests that, rather than an intravascular formation of platelet plug, a release of secondary mediators from these cells plays a prominent role. The increased accumulation of platelets in the lung microvasculature, and its significant reduction by treatment with a PAF-receptor antagonist, suggests that the platelets activated by generation of cardiac PAF during reperfusion of ischemic heart are trapped mainly in the lung. This may explain the increase in RVP observed during reperfusion and its abrogation by treatment with SDZ 63-675. A transient accumulation of platelets in the lung microvasculature associated with lung he-

modynamic alterations were observed after PAF infusion in the rabbit.[78,79] This accumulation of platelets may depend not only on their aggregation, but also on changes in pulmonary vascular pressures and blood flow. These observations would imply that the primary action of PAF in the ischemic-reperfused rabbit heart is to activate platelets and neutrophils in coronary vasculature, with deterioration of coronary circulation, that favor progression of myocardial necrosis in the ischemic area. However, we failed to demonstrate 15 minutes as well as 2 hours after reperfusion a significant difference in the RMBF in the ischemic area between control and SDZ 63-675–pretreated rabbits. This unexpected result may depend on transient vasoactive phenomena not explored in our protocol. However, the role of intravascular recruited neutrophils in no-reflow phenomenon has been recently revisited.[80,81] In fact, neutrophil depletion fails to modify the no-reflow phenomenon or improve functional recovery after reperfusion, despite reduction of the ultimate size of necrosis.[82] These observations may suggest that factors other than RMBF are involved in the role of PAF in the progression of ischemic myocardial injury. These may include an interstitial edema due to an early increase in vascular permeability, or a direct role of PAF in favoring lethal cellular injury of myocardiocytes. The latter effect may depend on the ability of PAF itself to stimulate oxygen radicals production from a variety of cells,[40,41] or on a direct cytotoxic effect reported for high concentrations of this mediator.[83] Whether cytotoxic concentrations of PAF may be reached locally at the cellular level or PAF may stimulate generation of secondary mediators relevant for progression of cellular injury in the ischemic heart remains to be determined. The role of PAF in amplifying the ischemic injury of myocardium is also supported by the observation that PAF administration after coronary occlusion extends the necrotic area.[84]

In conclusion, the present study provides evidence that PAF receptor antagonist may markedly reduce the infarcted area when administered before reperfusion of the ischemic heart. These results suggest that PAF may mediate at least in part the reperfusion injury of the heart. Further studies are needed to define the primary events involved in PAF synthesis and the targets of its biologic activity in the ischemic-reperfused heart.

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