Platelet-Activating Factor Acetylhydrolase Prevents Myocardial Ischemia-Reperfusion Injury

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Background—Platelet-activating factor (PAF) is one of the most potent biological mediators of tissue injury. PAF acetylhydrolase (PAF-AH) is a recently isolated naturally occurring enzyme that hydrolyzes PAF and renders it inactive. We hypothesize that inhibition of PAF with PAF-AH will reduce myocardial ischemia-reperfusion (I/R) injury in vivo.

Methods and Results—The coronary ligation model was used in New Zealand white rabbits. The large branch of the marginal coronary artery was occluded for 45 minutes, followed by 2 hours of reperfusion. Fifteen minutes before reperfusion, animals were given either 2 mg/kg of vehicle or of PAF-AH. At the completion of 120 minutes of reperfusion, percentage of necrosis, degree of neutrophil infiltration, and measurements of regional contractility were assessed. Data are expressed as the mean±SEM and compared by Student’s t test or Mann-Whitney ANOVA. Both groups of animals showed an equivalent area at risk; however, 46.7±11% was necrotic in the animal treated with vehicle. In contrast, 20.9±7.0% was necrotic in the animals treated with PAF-AH (P<0.05). Systolic shortening and wall thickness were significantly greater in those animals treated with PAF-AH at 15, 30, 60, and 120 minutes of reperfusion (P<0.05). Quantification of neutrophil infiltration showed a 62% reduction in the PAF-AH treated animals compared with those treated with vehicle alone.

Conclusions—PAF-AH is a potent cardioprotective agent in an in vivo model of I/R injury. (Circulation. 1999;100[suppl II]:II-365–II-368.)

Key Words: endothelium-derived factors ■ ischemia ■ reperfusion ■ myocardial infarction

This year, as many as 1.5 million Americans will have a new or recurrent myocardial infarction, and as a direct result, one third of these people will die. Although several types of interventions exist to treat this disorder, including PTCA and CABG, significant potential still exists for morbidity and mortality in the form of myocardial ischemia-reperfusion (I/R) injury. When the heart is rendered ischemic, a reduction occurs in the natural antioxidant defenses of the cells. At reperfusion, an accumulation of reactive oxygen intermediates results in a change in the redox potential of the cell, known as oxidative stress. Oxidative stress initiates a host inflammatory response characterized by widespread neutrophil adhesion and activation, initiation of coagulation, and alteration in vascular permeability.

Platelet-activating factor (PAF) is a potent biological mediator of inflammation and has been shown to participate significantly in I/R injury. Under normal physiological conditions, PAF is minimally expressed; however, under conditions of oxidative stress, as occurs in I/R injury, PAF is released by neutrophils and monocytes and is expressed on the outer leaflet of endothelial cells. Once expressed, PAF results in several diverse biological activities, including neutrophil activation and chemotaxis, alterations in vascular permeability, platelet activation, and negative inotropy, all of which may contribute to the clinical manifestations of I/R injury. The tissues, blood cells, and plasma of the body all have enzymes that catalyze the hydrolysis of PAF. This self-protective mechanism limits the damaging effect of PAF. Studies with newly available human recombinant PAF-acetylhydrolase (AH) have shown that it reduces edema, prevents asthma-related symptoms, and protects animals from septicemia. The protective effects of PAF-AH have never been studied under conditions of myocardial oxidative stress. The purpose of the present study was to test the hypothesis that PAF-AH reduces myocardial I/R injury.

Methods

In this model, adult New Zealand White rabbits weighing 3 to 4 kg each were used in research protocols approved by the Animal Care Committee of the University of Washington, Seattle. All animals received humane care according to the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 85-23).

Reagents

PAF-AH and the vehicle in which it was dissolved were acquired from ICOS Corp (Bothell, Wash). Both compounds, at a dose of 2 mg/kg, were given intravenously through the marginal ear vein 15 minutes before reperfusion. Twelve animals received PAF-AH: 6 were used for infarct data and 6 for histology. Twelve animals...
received vehicle: 6 were used for infarct data and 6 for histology. To ensure that recombinant PAF-AH alone had no effect on study end points, 3 animals received PAF-AH without ischemia; all were used for regional contractility data and histology.

**Surgical Procedure**

Rabbits were anesthetized with an initial intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). The rabbits were endotracheally intubated and maintained on inhaled halothane (1% to 2%) anesthesia in 100% O₂ using a small-animal respirator. A 20-gauge flexible catheter was placed in the left carotid artery to measure heart rate (HR) and mean arterial pressure and to collect blood samples. The heart was exposed via median sternotomy. An 8 F Millar catheter was placed through a small purse-string incision in the left ventricle to allow estimation of the left ventricular peak systolic pressure, left ventricular end diastolic pressure, and maximum and minimum change in left ventricular pressure (dP/dt). One pair of sonometric piezoelectric crystals was placed on the surface of the heart to assess regional wall motion abnormalities via segmental shortening (SS) in the area at risk. Another crystal was tunneled tangentially to the subendocardium, with a second crystal sutured to the epicardium for measurements of LV wall thickness (WT) (Sonometrics, Inc.). The hemodynamic trace acquired from the Millar catheter allowed SS and WT measurements to be correlated with end-diastolic and end-systolic time points. A 4.0 Vicryl suture was passed twice around a large anterolateral branch of the left main coronary artery that supplied to most of the left ventricle, and the ends of the suture passed through a small length of polyethylene tubing to form a snare. After a 20- to 30-minute stabilization period, baseline values were recorded. Regional myocardial ischemia was produced by reversibly occluding the artery for 45 minutes, followed by 120 minutes of reperfusion. Fifteen minutes before reperfusion, the rabbits received 2.0 mg/kg of PAF-AH or vehicle intravenously. Heart rate, mean arterial pressure, left ventricular end-systolic pressure, left ventricular end-diastolic pressure, ±dP/dt, WT, and SS were recorded at baseline: 30 minutes of ischemia; 45 minutes ischemia; and 15, 30, 60, and 120 minutes after reperfusion. Animals that received PAF-AH alone had the same measurements recorded at baseline and 15, 30, 60, and 120 minutes after drug administration. After 120 minutes of reperfusion, all animals were euthanatized with an intravenous bolus of concentrated pentobarbital, and the myocardial tissue was taken and processed for calculation of infarct size or histologic analysis.

**Determination of Infarct Size**

At the completion of the 120-minute reperfusion period, the coronary artery was reoccluded and 6 mL of 20% Evans blue dye (Sigma Chemical Co) was injected into the left atrium and allowed to circulate and stain all perfused tissue. The area supplied by the ligated vessel remained unstained, which demarcated the myocardium at risk of infarction. After the heart was arrested with pentobarbital, and the myocardial tissue was taken and processed for TTC staining. TTC stains the viable tissue brick red, which leaves the necrotic zone unstained. The tissue is then dehydrated and embedded in paraffin, and 3-mm sections were placed on glass slides. The slides were stained with hematoxylin and eosin. A pathologist blinded to the study protocol counted the number of neutrophils in 10 randomly selected fields (2200 μm²).

**Statistical Analysis**

The data analysis was performed using SPSS for Windows, version 6.1. For each of the treatment groups, several parameters (expressed as continuous variables) were studied at the indicated time points. To determine whether a statistically significant change had occurred compared with the baseline value, the parameters were compared with baseline by use of paired t tests. For intergroup comparisons, the mean change (from baseline) for each of these parameters was analyzed at the specific time points using ANOVA. The Mann-Whitney test was used for post hoc comparisons. A probability value of <0.05 was considered statistically significant.

**Results**

PAF-AH Reduces Infarct Size

The percentages of areas at risk for infarction were similar in both groups (vehicle, 44.3 ± 4.3%; PAF-AH, 46.73 ± 2.7%) as depicted in Figure 1. However, infarct size was markedly reduced in the group treated with PAF-AH (20.97 ± 3.3%) compared with the vehicle-treated group (45.72 ± 6.5%), P < 0.05.

PAF-AH Reduces Regional Myocardial Wall Motion Abnormalities

**Wall Thickness**

A similar reduction in WT after coronary occlusion occurred in both vehicle and PAF-AH groups (34% vehicle versus 49% PAF-AH group). By 120 minutes after reperfusion, the vehicle-treated animals had recovered to an average of only 45% of the baseline value, whereas the PAF-AH–treated group had recovered to an average of 85% of the baseline value (Figure 2). Animals that received PAF-AH alone maintained nearly 100% of the baseline value throughout the entire sampling period (data not shown).

**Systolic Shortening**

Coronary occlusion similarly reduced systolic shortening to 50% and 75% of baseline values in the vehicle and PAF-AH treated groups, respectively. However, after 120 minutes of reperfusion, the vehicle group had recovered only 54.5% of its baseline regional contractile function, whereas the PAF-AH group recovered to 103% of its baseline value (Figure 2). Animals that received PAF-AH alone maintained 100% of the baseline value throughout the sampling period (data not shown).

PAF-AH Reduces Neutrophil Infiltration

Histologic examination of myocardial tissue after I/R in the area at risk for infarction, revealed marked endothelial-leukocyte adhesion and transendothelial migration in those
animals receiving vehicle alone. In PAF-AH-treated animals, in contrast, neutrophil adherence was markedly reduced. Animals treated with vehicle alone had 157 polymorphonuclear neutrophils (PMNs) per high-powered field compared with 75 PMNs per high-powered field in PAF-AH–treated animals. Animals that received PAF-AH in the absence of ischemia averaged only 1 PMN per high-powered field (data not shown). These results suggest that PAF-AH inhibited neutrophil–endothelial cell interactions in vivo, although the difference in neutrophil accumulation was not significant (Figures 3 and 4). The enzyme alone appears to have no effect on neutrophil activation and migration, which correlates with unpublished data in the lung, kidney, and pancreas (Brian Schimpf, PhD, personal communication with ICOS, 1999).

**Discussion**

These results suggest that PAF plays a role in the regional cardiac dysfunction associated with I/R injury. The administration of its natural inhibitor, PAF-AH, markedly reduces this injury. Specifically, PAF-AH reduces infarct size and enhances regional ventricular functional recovery after I/R.

Several mechanisms exist by which PAF-AH may reduce I/R injury. One of the cardioprotective features of PAF-AH may stem from its ability to modulate leukocyte-endothelium inter-
actions. The endothelial cell, when exposed to conditions of oxidative stress, synthesizes PAF within minutes and transports it to the plasma membrane, at which it is expressed on the outer leaflet of the plasma membrane. Once expressed, PAF serves as both a chemoattractant and activator of neutrophils, which results in neutrophil-endothelial adherence, superoxide anion release, and degranulation.\(^2\)\(^3\) Furthermore, PAF may also result in vasconstriction,\(^5\) alteration in vascular permeability,\(^6\)\(^7\) and negative ionotropy\(^8\) in the setting of I/R. Any of these biological activities could explain the beneficial effect that exogenous administration of PAF-AH has in our model.

Much of the evidence implicating the role of PAF in I/R injury has been acquired through the use of PAF antagonists. PAF antagonists used in vivo models of I/R have proved to be cardioprotective, as indicated in functional measurements such as pressure-volume loops in swine,\(^9\) \(dP/dt\) in sheep,\(^10\) and systolic developed pressure in rabbits.\(^11\) Their use has also resulted in reduction of infarct size in the reperfused myocardium and inhibition of reperfusion-induced coronary artery vasoconstriction.\(^12\) Although these antagonists have proven to be a valuable research tool for elucidation of the role of PAF in I/R injury, they have also proven to be problematic when used clinically. Their detrimental effects include PAF agonist activities at high concentrations, antagonism of ADP and collagen production, significant hemolysis, and vascular damage.\(^2\)

In 1995, the cDNA encoding the plasma form of PAF-AH was cloned.\(^3\) The primary structure of plasma PAF-AH is unique. Its active site contains a catalytic triad found in many phospholipases that contains a serine, an acidic (usually aspartate), and a histidine residue. This catalytic triad hydrolyzes the acyl group at the second position of glycerol moiety. However, PAF-AH, unlike other phospholipases, has marked selectivity for phospholipids with short acyl chains at the sn-2 positions.\(^7\) Thus, normal membrane phospholipids are protected from hydrolysis by an intrinsic property of the enzyme. This same selective activity highlights its ability to be the most specific inhibitor of PAF known. As a result, this recombinant enzyme lacks the detrimental, non-selective activity of other PAF antagonists.

Early experience with PAF-AH in clinical trials has shown it to be a safe and efficacious means to treat inflammatory-mediated pulmonary dysfunction. Additionally, an increase in PAF levels has been documented during cardiac surgery with cardiopulmonary bypass. This finding did not correlate with a difference in serum PAF-AH activity.\(^13\) Therefore, exogenous administration of PAF-AH may prove to be a viable clinical means to reduce the detrimental activity of excessive PAF, which could result in a reduction in both myocardial injury and the generalized inflammatory consequences of cardiopulmonary bypass.

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