Iloprost Inhibits Neutrophil Function In Vitro and In Vivo and Limits Experimental Infarct Size in Canine Heart

Paul J. Simpson, Judith Mickelson, Joseph C. Fantone, Kim P. Gallagher, and Benedict R. Lucchesi

The prostacyclin analogue iloprost (ZK 36374) inhibits neutrophil activation in vitro, reduces neutrophil accumulation in inflammatory skin lesions, and reduces ultimate infarct size in an anesthetized open-chest canine model of regional ischemia and reperfusion. Iloprost (0.1-100 μM) inhibited the in vitro production of superoxide anion by canine neutrophils in a concentration-dependent manner. Iloprost (100 ng/kg/min i.v.) inhibited C5a-induced neutrophil migration into inflammatory skin lesions as assessed by the neutrophil-specific enzyme marker, myeloperoxidase. The myeloperoxidase activity determined 2 hours after the intradermal administration of C5a in each of the groups was control 13.3 ± 1.8 units/g tissue (n = 12) and iloprost 6.5 ± 0.9 units/g (n = 12), p < 0.01. Iloprost was administered to anesthetized open-chest dogs (100 ng/kg/min) 10 minutes after left circumflex coronary artery (LCCA) occlusion and continued during the 90-minute occlusion period and the first 2 hours of reperfusion. Regional myocardial blood flow was similar between treatment groups at baseline, 5 minutes and 80 minutes after LCCA occlusion, and after 1 hour of reperfusion. Infarct size, assessed 6 hours after reperfusion, was reduced by iloprost treatment: 22.4 ± 3.1 % of the area at risk (n = 15) compared with 42.4 ± 3.3 % of control (n = 13), p < 0.01. Iloprost treatment reduced the accumulation of neutrophils (measured by myeloperoxidase activity) in the ischemic myocardium at the interface between infarcted and noninfarcted tissue: control (n = 9) 9.0 ± 1.8 units/g tissue, iloprost (n = 6) 2.0 ± 0.4 units/g, p < 0.01. The ability of iloprost to reduce infarct size may be related both to a reduction in arterial blood pressure and to a modulation of neutrophil infiltration and activation at the site of tissue injury. (Circulation Research 1987;60:666-673)

There have been numerous attempts to modify the progression of myocardial tissue injury to irreversible injury and necrosis after regional myocardial ischemia and subsequent reperfusion. The damage that occurs on reperfusion is caused in part by an inflammatory response that leads to elaboration of mediators and accumulation of polymorphonuclear leukocytes (neutrophils). The protective effect of the nonsteroidal, anti-inflammatory agent ibuprofen in reducing infarct size is probably due to inhibition of neutrophil infiltration into the ischemic, reperfused myocardium. Neutropenia induced with specific neutrophil antibodies or with nitrogen mustard is associated with myocardial salvage in animal models of regional ischemia followed by reperfusion. Therefore, a potential pharmacologic approach for reducing ultimate infarct size and especially for reducing reperfusion injury would involve a reversible suppression of the associated inflammatory response or prevention of the formation and/or release of neutrophil derived cytotoxic products.

The arachidonic acid derived prostanoct, prostacyclin (PGI2), reduces reperfusion injury as assessed by a reduction in ultimate infarct size through a mechanism related to the inhibition of neutrophil migration and the inhibition of superoxide anion production. Moreover, the protective effects of PGI2 are independent of the degree of regional myocardial ischemia. Although effective in protecting the reperfused myocardium, the chemical instability of PGI2 in solution or in biologic fluids makes it a less than ideal agent for such application. The development of stable analogues of PGI2 offers the possibility that such agents can be employed during the management of patients with evolving myocardial infarction who are considered to be candidates for myocardial reperfusion.

The present report describes the effectiveness of the PGI2 analogue iloprost (ZK 36374; 5-(E)-(S,5S,6R,7R)-7-hydroxy-6-(E)-(3S,4RS)-3-hydroxy-4-methyl-1-octen-6-inyl) bicyclo [3.3.0] octan-3-ylidenepentanoic acid) in reducing myocardial infarct size after temporary regional myocardial ischemia. The mechanisms for the protective effect include reducing myocardial oxygen demand through systemic vasodilation and decreasing the neutrophil-mediated inflammatory response to ischemic myocardial injury.
Materials and Methods

General Preparation

Details of the methods have been published previously.3,6 Briefly, adult male mongrel dogs (12–17 kg) were anesthetized with Dial urethane (0.6 ml/kg) and ventilated with room air. The proximal left circumflex coronary artery (LCCA) was isolated and instrumented for continuous blood flow measurement with a calibrated electromagnetic flow probe. Arterial blood pressure, heart rate, the lead II electrocardiogram, and LCCA blood flow were recorded continuously.

Regional myocardial ischemia was produced by occluding the LCCA for 90 minutes and then reperfusing the vessel in the presence of a critical stenosis.7,8 The critical stenosis consisted of a silk ligature placed around the vessel and tightened just enough to reduce the reactive hyperemia by 60% of the prestenotic value after a 10-second mechanical occlusion. This stenosis prevented the development of hemorrhagic infarction and reduced the incidence of reperfusion-induced ventricular fibrillation. Reperfusion was maintained for 6 hours, after which the heart was fibrillated electrically, and the resulting infarct size was assessed by the ex vivo dual perfusion histochemical staining technique described by Romson et al.3 The histochemical staining method involved the perfusion of the cannulated LCCA with 1.5% triphenyltetrazolium chloride (TPT) solution buffered with 20 mM potassium phosphate (pH 7.4), while simultaneously perfusing the remainder of the coronary circulation with Evans blue dye introduced into the aorta. Both solutions were delivered to the respective vascular distributions under a constant pressure of 100 mm Hg and at a temperature of 39°C for a period of 5 minutes. The hearts were then cut into 5 or 6 transverse sections 1 cm thick, and infarct size was determined planimetrically. The method of quantifying infarct size with TPT has been validated by a number of investigators and has been shown to demarcate viable from nonviable myocardial tissue accurately as determined by the histochemical reaction between TPT and myocardial dehydrogenase enzymes.9–11

Two experimental treatment groups were studied: Group 1, controls (n = 16), received 0.9% saline, which was the vehicle for the drug-treated group; Group 2 (n = 19) received iloprost (Berlex Laboratories, Inc., Cedar Knolls, N.J.) at a dose of 100 ng/kg/min as an infusion administered via an intraluminal cannula. The infusion of iloprost was started 5 minutes after LCCA occlusion and continued throughout the 90 minutes of regional ischemia and during the first 2 hours of reperfusion.

Determination of Regional Myocardial Blood Flow

Regional myocardial blood flow was determined with tracer-labelled microspheres (15 μm diameter, New England Nuclear, Boston, Mass.) by the reference withdrawal method12 described previously.6,13 Four injections of microspheres (labelled with 141Ce, 113Sn, 103Ru, or 46Sc) were made in each experiment with the order of the isotopes being randomized. Reference arterial blood samples were obtained simultaneously from both the femoral and carotid arteries at a constant rate with a Harvard withdrawal pump, beginning immediately before the injection of microspheres into the left atrium and ending 2 minutes later. The reference sample counts were averaged for calculation of myocardial blood flow. If the reference sample counts varied by more than 15%, the data were discarded. Each bottle of microspheres was placed in an ultrasonic bath with subsequent vortex agitation before injection to insure that adequate dispersal of the microsphere suspensions was achieved before being administered.

The times of microsphere injection were as follows: Baseline flows were determined before occlusion. Myocardial blood flows were determined 5 minutes after LCCA occlusion, which was before drug or vehicle infusion was begun, 80 minutes after LCCA occlusion, and 1 hour after the initiation of LCCA reperfusion. Tissue samples weighing 0.5–1.0 g were dissected from the subepicardium, midmyocardium, and subendocardium sections of the heart in the LCCA perfused region and from the nonischemic region of the left ventricle. At least 3 sections from each heart were used so that blood flows to each region represent the average of 3 or 4 samples for each experiment.

Evaluation of Neutrophil Accumulation in Myocardial Tissue

Samples of myocardium (50–200 mg) were taken from the central infarct region, the noninfarcted tissue within the area at risk, the endocardial-to-epicardial border zone between infarct region and area at risk (Figure 1), and from normal noninfarcted and unstained myocardium. The tissue samples were homogenized and assayed for myeloperoxidase content as described.14 The myocardial content of myeloperoxidase activity has been correlated with histologic evidence of neutrophil infiltration.15,16

![Figure 1. Method for the quantification of myocardial infarct size expressed as percent of area at risk and as percent of total left ventricle after histochemical demarcation of the respective myocardial regions.](http://circres.ahajournals.org/doi/abs/10.1161/01.CIR.84.7.667? ferach=true)
Evaluation of Neutrophil Migration In Vivo

Four dogs were anesthetized with Dial urethane and randomized into each of 2 treatment groups: One group, which served as controls (n = 2), received 0.9% sodium chloride while the second group (n = 2) received iloprost at a dose of 100 ng/kg/min intravenously. The chest and back of each dog were shaved, and 0.1 ml zymosan-activated dog plasma (ZAP) was injected intradermally at random sites 30 minutes after the respective treatment regimens (drug or vehicle) had begun. ZAP was prepared by incubating normal dog plasma with 10 mg/ml of zymosan A (Sigma Chemical Co.) for 30 minutes at 37° C and then removing the zymosan by centrifugation. Zymosan added to plasma results in the activation of the complement system. The complement component, which is responsible for neutrophil activation, is C3. The full-thickness skin biopsies (100–300 mg each) were taken at 0, 1, and 2 hours after ZAP injection. The neutrophil-specific enzyme, myeloperoxidase, was extracted from the biopsies and measured spectrophotometrically. The myeloperoxidase content of dermal tissue is an accurate marker of neutrophil accumulation.

Evaluation of Effects of Iloprost on Neutrophil Superoxide Production In Vitro

Canine neutrophils were isolated from venous blood obtained from untreated dogs and separated by Ficoll-Hypaque (Histopaque 1077, Sigma Chemical Co.) gradient centrifugation followed by red blood cell lysis with buffered ammonium chloride [(NH₄)₂C₂O₄, 150 mM; NaHCO₃, 10 mM; ethylenediamine tetraacetic acid (EDTA), 1 mM; pH 7.2]. Cell preparations were greater than 95% neutrophils, and cell viability was greater than 90% as determined by the trypan blue exclusion method. Superoxide production was measured by a modification of the method of Babior et al as described by Fantone and Kinnes. Neutrophils (5 × 10⁶/ml) were incubated at 37° C in the presence of 0.1 mM ferricytochrome c and cytochalasin B (5 µg/ml) in Hanks’s balanced salt solution (HBSS) with 1 mg/ml glucose. After an initial 5-minute incubation, opsonized zymosan (OZ) (1 mg/ml final, 10% of final volume) was added to the tubes. OZ was prepared by incubating normal dog serum with zymosan A (10 mg/ml) for 30 minutes at 37° C and then washing the zymosan twice with sterile normal saline. The OZ was resuspended in normal saline and diluted to a concentration of 10 mg/ml. Superoxide production was determined as the superoxide dismutase (SOD) inhibitable reduction of ferricytochrome c to ferrocytochrome c at 550 nm. Iloprost was diluted serially in HBSS and added to the incubation tubes just before the addition of the neutrophils.

Statistical Analyses

All data were compared with respective control groups by Student’s t test except for the incidence of ventricular fibrillation, which was compared between groups with a χ² test. Values of p < 0.05 were considered significant.

Results

Reduction of Myocardial Infarct Size

A total of 35 dogs was included in the study to determine the effects of iloprost on the size of myocardial infarcts resulting from occlusion of the LCCA for 90 minutes followed by reperfusion for 6 hours. Three dogs that had been treated with vehicle (control) and 4 dogs that had been treated with iloprost were excluded from the study because of ventricular fibrillation. These dogs were not successfully defibrillated (fewer than 4 attempts at DC cardioversion). Myocardial infarct size expressed as a percentage of the area of the left ventricle at risk of infarction (IN/AR) is depicted in Figure 2. Iloprost produced a 47% decrease in IN/AR compared with control: control (n = 13), IN/AR 42.4 ± 3.3%; iloprost (n = 15), IN/AR 22.4 ± 3.1%; p < 0.01. Infarct expressed as a percentage of the total left ventricle (IN/LV) was similarly decreased by iloprost treatment: control (n = 13), IN/LV 20.0 ± 1.7%; iloprost (n = 15), IN/LV 9.8 ± 1.4%; p < 0.01. The percentage of left ventricle that was rendered ischemic and constituted the area at risk (AR/LV) was similar in magnitude in each of the groups: control (n = 13), AR/LV 47.4 ± 1.8%; iloprost (n = 15), AR/LV 43.9 ± 1.6%.

Hemodynamic Data

Heart rate (HR), mean arterial pressure (MAP), and the rate pressure product (RPP) measured during the course of the experiments are depicted in Table 1. RPP (systolic blood pressure × heart rate/1,000) is used as an index of myocardial oxygen consumption. Baseline HR, MAP, and RPP were similar in control and iloprost-treated groups. However, during regional myocardial ischemia, HR, MAP, and RPP were significantly less with iloprost treatment than with control. The vasodilator effects of iloprost are reflected in a lower MAP and RPP in the iloprost-treated group compared with the control group during the first 2 hours of reperfusion. Iloprost prevented the reflex increase in heart rate that is normally associated with
decreases in blood pressure. This preventive action has been demonstrated before with prostacyclin and has been attributed to activation of vagal depressor reflexes.21 After 2 hours of reperfusion, infusions were stopped, and the MAP increased significantly there-after in the iloprost-treated group compared with a decrease in MAP of the control group. The RPP was significantly less with iloprost during the first hour of reperfusion but was similar to control during the remaining 6 hours of reperfusion. There were no significant differences between treatment groups in LCCA blood flow (Table 2).

**Regional Myocardial Blood Flow**

There were no significant differences in regional myocardial blood flow (RMBF) between treatment groups at baseline, early or late occlusion or at 1 hour of reperfusion (Figure 3). Regional blood flow to the ischemic region was reduced to 3% of baseline in the subendocardium, 7% of baseline in the midmyocardium, and 20% of baseline in the subepicardium in the vehicle-treated group. In the iloprost-treated group, RMBF was reduced to 4%, 7%, and 16% of baseline in subendocardium, midmyocardium, and subepicardium, respectively.

Endocardial/epicardial blood flow ratios (ENDO/EPI) within the ischemic myocardium were as follows:

- Baseline period: control group 1.37 ± 0.08, iloprost group 1.37 ± 0.06.
- Early occlusion period: control group 0.24 ± 0.04, iloprost group 0.34 ± 0.06.
- Late occlusion period: control group 0.21 ± 0.06, iloprost group 0.18 ± 0.05.
- Reperfusion period: control group 1.32 ± 0.26, iloprost group 1.24 ± 0.35.

There were no significant differences in the END/EPI ratios between treatment groups.

**Myocardial Myeloperoxidase as an Index of Neutrophil Accumulation**

Myeloperoxidase activity in tissue taken from the center of the infarct in control hearts was 9.0 ± 3.3 units/g. Treatment with iloprost resulted in a significant reduction (78%) in neutrophil accumulation in the border region between infarcted and noninfarcted tissue (p < 0.005) and also within the area of the myocardium at risk of infarction (77% reduction, p < 0.05) (Figure 4). Although the mean value for myeloperoxidase content in the central infarct region was less in the iloprost group, it failed to reach statistical significance. The myeloperoxidase content of noninfarcted tissue taken from normal myocardium was 0.2 ± 0.1 units/g (n = 10), indicating a paucity of neutrophils in the noninfarcted myocardial regions of the left ventricle.
Inhibition of Neutrophil Superoxide Anion Production In Vitro by Iloprost

There was a concentration-dependent inhibition by iloprost of the in vitro neutrophil superoxide anion production (Figure 5). Superoxide anion production in the absence of iloprost was 10.42 ± 0.40 nmol/10 min/5 x 10^6 cells. Incubation of the neutrophils in the presence of iloprost at a concentration as low as 0.1 μM produced a significant inhibition (p < 0.05) in superoxide anion production.

Dermal Neutrophil Accumulation

Intradermal injection of zymosan-activated plasma produced a time-dependent accumulation of neutrophils within the inflammatory skin lesion (Figure 6). Intravenous infusion of iloprost at the same dose that reduced myocardial infarct size (100 ng/kg/min) reduced the accumulation of neutrophils at the site of the skin lesion by 51% compared with controls (p < 0.001) as assessed by the content of myeloperoxidase present at the site of the dermal lesion 2 hours after the injection of ZAP.

Incidence of Ventricular Fibrillation

The overall incidence of ventricular fibrillation (VF) was 14/35, with the control incidence 8/16, and the iloprost-treated group 6/19 (p < 0.05). Therefore, there were no differences in the frequency of VF between the 2 treatment groups.
the infarcted region, especially in the border zone between irreversibly injured and viable myocardium.

The mechanisms by which iloprost affords protection to the regionally ischemic, reperfused heart in this experimental model are probably both due to a reduction in myocardial oxygen demand and to a prevention of neutrophil-mediated damage. Iloprost has been reported to reduce myocardial ischemic damage after permanent coronary ligation in the cat,22 after coronary artery microembolization in the pig,23 and after coronary ligation in the rat.24 The mechanism for protection by iloprost in vivo has been attributed to a reduction in arterial blood pressure,22 an increase in collateral blood flow to the ischemic myocardium,23 prevention of destruction of adrenergic nerve terminals within the myocardium,23 or to a "membrane stabilizing" effect.22

Altered Neutrophil Migration Into Ischemic Myocardium

It is becoming increasingly evident that the myocardial injury that occurs during regional ischemia and reperfusion is exacerbated by the infiltration of neutrophils into the previously ischemic myocardial region or area at risk. The role of neutrophils as inflammatory cells during myocardial ischemia and reperfusion has been demonstrated by a number of studies in which neutropenia,2-4 anti-inflammatory agents,2-4 prostaglandins,26 or free radical scavengers27 reduced the ultimate size of infarction while reducing the neutrophilic infiltrate into the myocardium. This subject has been reviewed recently.1 The present report demonstrates that neutrophil function is altered in vitro as determined by superoxide production (Figure 5) and in vivo as assessed by neutrophil accumulation in the myocardium (Figure 4). Iloprost was effective in reducing the neutrophil accumulation within the myocardium at the border zone between infarcted and noninfarcted tissue. Mullane et al16 observed that after 90 minutes of coronary occlusion and 5 hours of reperfusion, neutrophil accumulation was greatest at the interface between infarcted and normal myocardial tissue. These observations suggest that this interface may be the site of reversible myocardial injury and the area of the most intense inflammatory response. As such, the endocardial-to-epicardial border zone may be most amenable to treatment by agents that produce an anti-inflammatory effect through a modulation of neutrophil function, that alter neutrophil chemotaxis, or that counteract the cytotoxic products released by the activated inflammatory cells.

Altered Myocardial Oxygen Supply or Demand

Maroko et al24 suggested that a reduction in blood pressure would protect ischemic myocardium by reducing myocardial oxygen demand. In the present study, reduced myocardial oxygen demand cannot be excluded as a possible protective mechanism. However, in the cat22 and in the rat,24 myocardial protection was demonstrated to be independent of effects on blood pressure since a blood pressure reduction was not observed at the doses used. Moreover, Chiariel et al26 demonstrated that a reduction in blood pressure alone was not sufficient to produce myocardial protection. When nitroglycerin and nitroprusside were compared in open-chest anesthetized dogs, both drugs produced a reduction in mean arterial blood pressure, but only nitroglycerin reduced ischemia by increasing blood flow to the ischemic myocardium as measured with radiolabelled microspheres. Furthermore, Jugdutt et al30 demonstrated that the protective effect of nitroglycerin in conscious dogs was due to the direct coronary vasodilating effects of the drug and did not depend on the reduced arterial blood pressure. The protective effects of reduced preload and afterload were dependent on the increased perfusion of the ischemic myocardium. Likewise, subsequent studies by Jugdutt et al31 confirmed these observations. Studies with PGI2, PGE1, and PGE3 on ultimate infarct size in the canine heart subjected to permanent coronary artery occlusion31 demonstrated that all 3 prostaglandins reduced blood pressure, but only PGI2 and PGE1 reduced infarct size and increased collateral blood flow to the ischemic region. Thus, the protection in those experimental models was not dependent on a reduction in blood pressure but instead on the increased blood flow to the ischemic myocardium. Regional myocardial blood flow in the present study with iloprost demonstrated that there were no effects of treatment on collateral blood flow during occlusion or on reperfusion. Thus, protection was not due to increasing collateral flow in the iloprost group, nor was it due to prevention of the "no reflow phenomenon."32

Simpson and coworkers,9 using PGI2 and a stable PGI2 analogue (SC39902), demonstrated that with temporary regional myocardial ischemia followed by reperfusion, PGI2 reduced infarct size but the analogue (SC39902) proved to be ineffective. Since both agents reduced blood pressure to the same extent and collater-
al blood flow to the ischemic myocardium was not increased with PGI$_2$ treatment, the protective effect of PGI$_2$ was attributed to inhibition of neutrophil function. The PGI$_2$ analogue (SC39902) did not inhibit neutrophil activation, but PGI$_2$ produced potent and effective inhibition of neutrophil activation in vitro and reduced the accumulation of neutrophils in vivo in inflammatory skin lesions. Thus, the protective effect of PGI$_2$ with reperfusion of the ischemic myocardium is due to the anti-inflammatory effects on neutrophils.

The antiplatelet effects of iloprost have been demonstrated. Iloprost is similar in potency and efficacy to prostacyclin with respect to inhibition of platelet activation. It would seem to be of at least theoretical benefit during ischemia and reperfusion to prevent platelet aggregation and "plugging" of the coronary microvasculature. However, the recent studies by Jolly et al$^{24}$ and Bednar et al$^{25}$ suggest that the platelet plays a rather passive role during the process of infarction, accumulating in already damaged tissue.

The present study provides direct evidence of an altered functional response of neutrophils in vivo by iloprost at a dose that reduces myocardial infarct size. Since dermal neutrophil migration in vivo was reduced, it seems likely that the decreased myocardial accumulation of neutrophils, as determined by the presence of myeloperoxidase activity, is a direct effect of iloprost on neutrophil infiltrative function and is not a result of reducing myocardial infarct size itself. Furthermore, iloprost has direct inhibitory effects on neutrophil function as measured by its ability to reduce superoxide anion production. These data suggest that the protective effect of iloprost on infarct size is due to reduced neutrophil function as well as reduced arterial blood pressure.

It has been previously reported that lower doses of iloprost and prostacyclin have antiarrhythmic properties when used against ischemia and reperfusion arrhythmias in dogs. The data from the present study refute those results concerning the effect of these agents on the incidence of ventricular fibrillation because there was no difference in the frequency of VF between control or iloprost treatment groups. Since the direct electrophysiologic effects of iloprost have not been demonstrated, the differences in arrhythmias between the two studies may be explained by differences in the severity of ischemia.

The effectiveness of iloprost in reducing myocardial ischemia and reperfusion injury suggests that it may be useful clinically for the treatment of patients with an evolving myocardial infarction. Iloprost is similar in potency and efficacy to prostacyclin for inhibiting platelet activation and reducing blood pressure. PGI$_2$ improves coronary blood flow after streptokinase thrombolysis, suggesting that iloprost has the potential to improve coronary blood flow after thrombolysis as well, while at the same time it may provide protection from "reperfusion injury." The data from the present study confirm the observations of others with respect to the myocardial protective effects of iloprost and also provide evidence that the protective effect is due to modification of neutrophil infiltration and activation after myocardial ischemia and reperfusion.

The time frame for drug infusion and the route of administration in the present study were chosen to maximize any beneficial effect. Future studies will determine whether iloprost is equally effective when administered at different times during occlusion and reperfusion.

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References

15. Bednar M, Smith B, Pinto A, Mullane KM: Nafazatrom-in-


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