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Prostacyclin protects ischemic reperfused myocardium in the dog by inhibition of neutrophil activation

Prostacyclin (PGI_2) and the stable PGI_2 analogue SC39902 (6,9α-epoxy,5S-fluoro-11α,15S-dehydroxyprosta-6,13E-dien-1-oic acid, sodium salt) were studied in anesthetized open-chest dogs subjected to 90 minutes of left circumflex coronary artery (LCCA) occlusion and 6 hours of reperfusion. PGI_2 (50 ng/kg/min, infused into the left atrium) reduced infarct mass by 59% compared to control, but SC39902 (1.5 μg/kg/min) failed to produce a significant reduction in infarct size. Both PGI_2 and SC39902 reduced mean arterial blood pressure, heart rate, and rate-pressure product to the same extent. Regional myocardial blood flow measured with radiolabelled tracer microspheres did not demonstrate an increase in regional blood flow to the ischemic myocardium during the 90 minutes of LCCA occlusion in the PGI_2 and control treatment groups. Canine neutrophils were isolated from whole blood and activated with opsonized zymosan. PGI_2 produced a concentration-dependent inhibition of neutrophil activation as measured by superoxide production in vitro, whereas SC39902 failed to effectively inhibit neutrophil activation. Neutrophil migration into inflammatory skin lesions was effectively attenuated when dogs were pretreated with PGI_2 (50 ng/kg/min, intravenously). Therefore, it is suggested that the cytoprotective effect of PGI_2 during myocardial ischemia and reperfusion is related to an inhibition of neutrophil migration and the production of cytotoxic activated oxygen species. (Am Heart J 1987;113:129.)

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Prostacyclin (epoprostenol, PGI_2) is the major active arachidonic acid metabolite produced by vascular endothelium.[1,2] It is the most potent endogenous inhibitor of platelet aggregation yet described,[1] and is a potent systemic vasodilator[3] and coronary artery vasodilator.[4] Prostacyclin has been demonstrated to be protective in a number of experimental models of myocardial ischemia.[5-8] Lefer et al.[9] demonstrated

the usefulness of PGI_2 for the treatment of acute myocardial ischemia. This was based on the ability of PGI_2 to inhibit platelet aggregation and favorably alter myocardial oxygen supply and demand by increasing coronary artery blood flow and reducing systemic blood pressure, respectively. Melin and Becker[10] demonstrated that PGI_2 protects in the absence of an increase in collateral blood flow to the ischemic myocardium.

Recent studies[11-13] have provided substantial evidence to demonstrate that polymorphonuclear leukocyte (neutrophil) infiltration during an evolving myocardial infarct contributes to irreversible myocardial tissue injury. Since prostacyclin inhibits in vitro production of cytotoxic oxygen-free radicals and release of degradative lysosomal enzymes,[14-16] we hypothesize that the cytoprotective effect of PGI_2 in canine regional myocardial ischemia and reperfusion is at least partly due to inhibition of neutrophil activation.

The purpose of this investigation was to determine the effectiveness of PGI_2 and the chemically stable mono-fluorinated PGI_2 analogue (SC39902; 6,9α-epoxy,5S-fluoro-11α, 15S-dehydroxy-prosta-6,13E-dien-1-oic acid, sodium salt) for reducing the extent of irreversibly injured myocardial tissue that results after 90 minutes of regional ischemia and 6 hours of reperfusion. We report on the effects of PGI_2 and SC39902 on canine neutrophil activation by the particulate phagocytic stimulus, opsonized zymosan, as measured by superoxide anion production in vitro. Additionally, we report that PGI_2 inhibits neutrophil migration in vivo into inflammatory skin sites at the same dose that reduces myocardial ischemia-reperfusion injury.

METHODS

Details of the methods have been published previously.[12,17] Briefly, adult male mongrel dogs (12 to 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 an electromagnetic flow probe. Heart rate, arterial blood pressure, LCCA blood flow, and ECG were continuously recorded. The three experimental groups in the study were: (1) Control (vehicle) (n = 16), 50 mM Tris-HCl, pH 9.4 at 4° C in 0.9% saline; continuous infusion (0.5 ml/min) into the left atrium beginning 30 minutes before coronary artery occlusion, continuing during the 90 minutes of regional ischemia, and continuing during the first 2 hours of reperfusion. (2) Prostacyclin (n = 14), 50 ng/kg/min in the same vehicle, over the same time course as control. (3) SC39902 (G.D. Searle & Co.) (n = 10), 1.5 μg/kg/min (same vehicle and time course).

Regional myocardial ischemia was produced by occluding the LCCA for 90 minutes and then reperfusing through a critical stenosis[18] for 6 hours. After 6 hours the infarct mass was assesed with the ex vivo dual perfusion technique previously described,[12] in which the LCCA is perfused with 1.5% triphenyltetrazolium chloride in 50 mM potassium phosphate buffer (pH 7.4 at 37° C) and the remainder of the coronaries are perfused with 0.25% Evan's blue dye. The hearts were then cut into five or six 1 cm thick transverse sections. Infarct size was then determined planimetrically.

Regional myocardial blood flow (RMBF). Radioactive microspheres (15 μm) (New England Nuclear, North Billerica, Mass.) labelled with one of the following five isotopes—^{51}Cr, ^{141}Ce, ^{85}Sr, ^{103}Ru, ^{46}Sc—were used to assess regional myocardial blood flow,[19] essentially as detailed previously.[17] The times of microsphere injection were as follows: baseline (before drug or vehicle infusion), 25 minutes after drug or vehicle infusion, 10 minutes after LCCA occlusion, and 80 minutes after LCCA occlusion.

Tissue samples weighing 0.5 to 1.0 gm were dissected from sections of the hearts after staining, from subepicardium, midmyocardium, and subendocardium from the LCCA perfusion region (which was rendered ischemic), and from the normally perfused nonischemic region. Three sections from each heart were used so that blood flows to each region represent the average of three samples for each experiment.

Histology. A midventricular transmural section of the area of the left ventricle supplied by the LCCA was examined by light microcopy in 21 hearts, seven from each of the three treatment groups (control, PGI_2, SC39902). Paraffin-embedded tissue blocks were sectioned at 5 thickness and were stained with hematoxylin and eosin. The presence of necrosis was verified and the degree of hemorrhage was assessed by a pathologist (GDA) unaware of the experimental treatment groups.

Neutrophil preparation. Canine neutrophils were isolated from venous blood obtained from untreated dogs and were separated by Histopaque (Sigma Chemical Co., St. Louis, Mo.) gradient centrifugation followed by red blood cell lysis with a buffered ammonium chloride solution (150 mM $[NH_4]_2C1_2$; 10 mM $NaHCO_3$; 1mM EDTA; pH 7.2). Cell viability was greater than 90% as determined by trypan blue exclusion. Cell preparations consisted of greater than 95% neutrophils. Superoxide (O_2^-)production was measured by a modification of the method of Babior et al.[20] as described by Fantone and Kinnes.[14]

Neutrophils (5×10^6 cells/ml) were incubated at 37° C in the presence of 0.1 mM ferricytochrome C in Hanks' balanced salt solution (HBSS) with 1 mg/ml glucose. The rate of superoxide production was determined as the superoxide dismutase (SOD) inhibitable (SOD 50 μg/ml in reference tubes) reduction of ferricytochrome C to ferrocytochrome C with the use of an extinction coefficient of 21.1 mM^{-1} cm^{-1} at 550 mn. After an initial 3- minute incubation at 37° C, Cytochalasin B (5 μg/ml) was added and 2 minutes later, opsonized zymosan (OZ) (10% of final volume) was added to the neutrophils. OZ was prepared by incubation of normal dog serum with zymo-

Table I. Hemodynamic measurements

		Control (n = 16)	*Prostacyclin (n = 14)*	*SC39902 (n = 10)*
Heart rate (bpm)	Baseline	145 ± 6	124 ± 5†	138 ± 8
	Drug	148 ± 6	119 ± 5†	130 ± 9*
	Occlusion	139 ± 5	124 ± 5	139 ± 5
	Reperfusion	151 ± 8	148 ± 8	180 ± 13
Mean arterial pressure (mm Hg)	Baseline	119 ± 4	115 ± 5	119 ± 6
	Drug	119 ± 4	88 ± 5†	101 ± 5†
	Occlusion	93 ± 4	78 ± 4*	90 ± 3
	Reperfusion	91 ± 4	93 ± 5	102 ± 5
Rate–pressure product (mm Hg/min × 1000)	Baseline	21.6 ± 1.4	18.1 ± 1.2	20.1 ± 1.4
	Drug	22.7 ± 1.6	14.1 ± 0.9†	16.2 ± 1.2†
	Occlusion	16.7 ± 1.0	13.5 ± 1.0	16.1 ± 1.0
	Reperfusion	17.4 ± 1.8	18.5 ± 1.4	23.6 ± 2.0

All values expressed as mean ± standard error.
Baseline = before drug; Drug = 30 minutes after vehicle or drug infusion; Occlusion = 30 minutes after LCCA occlusion; Reperfusion = 6 hours after LCCA reperfusion.
*$p < 0.05$, †$p < 0.01$, compared to control at same time point.

san A (Sigma; 10 mg/ml) for 30 minutes at 37° C and then by washing the zymosan twice with sterile normal saline. The OZ was then resuspended in normal saline to 10 mg/ml. Prostaglandins, when present, were serially diluted immediately before use in HBSS (4° C) and were added at the same time as the cells.

Evaluation of neutrophil migration in vivo. Seven dogs were anesthetized with Dial-urethane and (randomized into one of two treatment groups: (1) Prostacyclin, 50 ng/kg/min in vehicle (50 mM Tris HCl buffer (pH 9.4 at 4° C) in 0.9% saline) by intravenous infusion 0.5 ml/min; or (2) Control (vehicle). The chest and back of each dog was carefully shaved and zymosan-activated dog plasma (ZAP) (0.2 ml) was injected intradermally at random sites 30 minutes after the respective intravenous treatment had begun. ZAP was prepared by incubating normal dog plasma with zymosan A (10 mg/ml) for 30 minutes at 37° C and then removing the zymosan by centrifugation. Biopsies (100 to 300 mg each) were taken at 0, 0.5, 1.0, and 2.0 hours after ZAP injection. The neutrophil-specific enzyme, myeloperoxidase[21] was extracted and measured spectrophotometrically as detailed by Bradley et al.[22] The myeloperoxidase content of tissue has been shown to be a good marker of skin neutrophil content.[21, 22]

Statistical analyses. Hemodynamic data (heart rate, mean arterial pressure rate-pressure product, LCCA blood flow) and infarct sizes were analyzed with a one-way analysis of variance (ANOVA) at each time point where there were three groups (control, PGI_2, and SC39902). The groups of data that were found to be significantly different from the ANOVA were analyzed further with Dunnett's test for multiple comparisons to control.[23] p values less than 0.05 were considered significant. Regional myocardial blood flows (RMBFs) were analyzed with unpaired Student's t tests for each comparison between the two treatment groups. Paired Student's t tests were used to test for differences with time in the RMBF studies.[24] A Bonferroni α-error protection was used if any of these differences were found to be significant. In vitro superoxide production was analyzed by unpaired Student's t test for each concentration of PGI_2 or SC39902 compared to the superoxide production in the absence of either PGI_2 or SC39902. Myeloperoxidase content of tissue samples were compared by Student's t test.

RESULTS

Thirty-four dogs were assigned randomly to one of the three treatment groups (control, PGI_2, or SC39902) for initial assessment of drug effect on resulting myocardial infarct size. Three dogs, one from each of the three treatment groups, were eliminated due to ventricular fibrillation during reperfusion which was not converted with fewer than four DC countershocks. Fourteen more dogs were randomized into one of two treatment groups (control or PGI_2) for assessment of treatment effects on changes in RMBF. Of these 14 dogs, three control dogs and one PGI_2-treated dog were eliminated from the study due to ventricular fibrillation. Two more dogs (one control, one PGI_2) were eliminated from the study due to technical problems with microsphere injection and reference blood sample withdrawals.

Hemodynamic effects of PGI_2 and SC39902. The doses of PGI_2 (50 ng/kg/min) and SC39902 (1.5 μg/kg/min) were chosen on the basis of their ability to produce a similar decrease in blood pressure. Prostacyclin infusion resulted in a 23% reduction (−27 mm Hg) in mean arterial blood pressure (MAP) (Table I) after 30 minutes of infusion, from

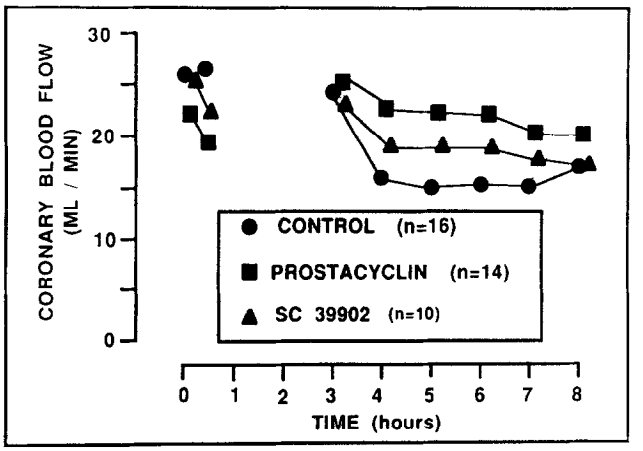

Fig. 1. Left circumflex coronary artery (LCCA) blood flow at regular intervals during the experiments. Drug or vehicle infusion was begun after recording 0 hour flows and was continued until 2 hours after reperfusion (4 hours). The LCCA was occluded for 90 minutes, beginning after 30 minutes of drug or vehicle infusion. There were no differences among treatment groups at any time point (ANOVA).

115 ± 5 to 88 ± 5 mm Hg (n = 14). Similarly, infusion of SC39902 resulted in a reduction in MAP from 119 ± 6 to 101 ± 5 mm Hg (n = 10). Occlusion of the LCCA typically results in a decline in MAP, as demonstratd in Table I comparing control before occlusion (119 ± 4 mm Hg) to control at 30 minutes of regional myocardial ischemia (93 ± 4 mm Hg). MAP was comparable after LCCA occlusion among all three treatment groups throughout the 6-hour reperfusion period.

Baseline heart rates (HR) (Table I) were slower in the PGI_2-treated group ($p < 0.05$); however, after 30 minutes of PGI_2 or SC39902 infusion, both PGI_2 and SC39902 groups had significantly slower heart rates compared to control (both $p < 0.05$). Heart rates were comparable among groups during the 90-minute ischemic period and throughout the 6-hour reperfusion period.

Rate–pressure product (RPP) (systolic blood pressure × heart rate/1000) is used as an index of myocardial oxygen consumption.[25] RPP was monitored throughout the experiments to determine whether changes in oxygen demand due to treatment could be responsible for any protective effect on the resulting myocardial infarct size. Baseline mean values for RPP did not differ among the three treatment groups (Table I) (Control: 21.6 ± 1.4 mm Hg/min/1000; PGI_2: 18.1 ± 1.2 mm Hg/min/1000; SC39902: 20.1 ± 1.4 mm Hg/min/1000). However, after 30 minutes of infusion of PGI_2 or SC39902, rate–pressure product was significantly reduced in both PGI_2 and SC39902 treatment groups compared to control ($p < 0.01$). Upon LCCA occlusion, this index was similar among groups and remained so throughout the 6-hour reperfusion period.

Mean LCCA blood flows were comparable before treatment (Fig. 1). After 30 minutes of infusion of PGI_2 or SC39902, LCCA coronary blood flows were similar when compared to the vehicle control group (control = 27 ± 3 ml/min, n = 16; PGI_2 = 19 ± 2 ml/min, n = 14; SC39902 = 22 ± 4 ml/min, n = 10; PGI_2 both $p > 0.05$ compared to control). After 90 minutes of complete LCCA occlusion, blood flow was resumed slowly over 15 minutes. All mean LCCA blood flows were comparable among groups at every time point observed subsequent to reperfusion.

Effects of PGI_2 and SC39902 on infarct size. The effects of PGI_2 and SC39902 on resulting myocardial injury due to ischemia and reperfusion are depicted in Fig. 2. The ratios of the amount of left ventricle rendered ischemic *(AREA AT RISK* [AR]) to the total left ventricle (LV) were equivalent among the three treatment groups (control: n = 13, AR/LV = 45.5 ± 1.7%, PGI_2: n = 12, AR/LV = 40.3 ± 2.4%; SC39902: n = 10, AR/LV = 48.9 ± 2.1%; $p > 0.05$ by ANOVA). Infarct size was reduced significantly with PGI_2 treatment ($p < 0.001$ by ANOVA and Dunnett's, control vs PGI_2) when expressed as a percentage of LV or as a percentage of the area at risk. On the other hand, SC39902 demonstrated a hemodynamic profile (HR, MAP, RPP, LCCA blood flow) similar to that with PGI_2 but failed to significantly alter the size of the myocardial infarct that evolved after 90 minutes of regional ischemia and 6 hours of reperfusion. Infarcts expressed as a percentage of the area of myocardium at risk were as follows: control: n = 13, 47.4 ± 4.7%; PGI_2: n = 12, 19.2 ± 2.0%; SC39902: n = 10, 36.5 ± 5.5%; ($p < 0.001$ Dunnett's, control compared to PGI_2).

Regional myocardial blood flows were determined at four time points during the experiments (Fig. 3). The purpose of measuring RMBF is to determine whether the protective effect of PGI_2 on the evolving mass of myocardial infarct is due to alteration of regional blood flow distribution. LCCA occlusion resulted in a significant decline in myocardial blood flow in all three myocardial regions (subepicardial, midmyocardial, and subendocardial) and to a similar extent in both control and the PGI_2 treatment groups. Endocardial blood flow was reduced by 91% and 93% in control and prostacyclin treated groups, respectively; midmyocardial flow declined by 84% (control) and 91% (PGI_2); and epicardial flows declined by 67% (control) and 85% (PGI_2). Blood flows in each of the three regions were not increased

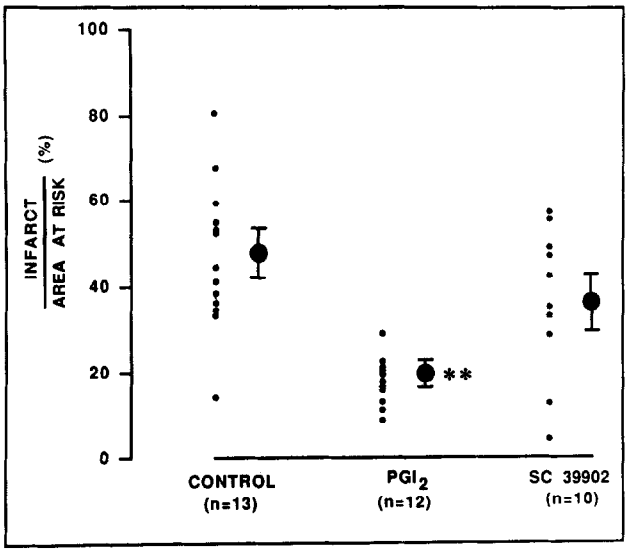

Fig. 2. Myocardial infarct size was measured after 6 hours of reperfusion. Mean infarct mass is expressed as a percentage of the area of the myocardium at risk of infarction, depicted as individual points for each experiment along with the mean ± standard error of the mean for each treatment group. PGI_2 treated dogs developed a significantly smaller infarct compared to controls (**$p < 0.01$, ANOVA, Dunnett's).

during LCCA occlusion in the ischemic perfusion bed when early (10 minutes after occlusion) and late occlusion (80 minutes after occlusion) flows were compared (paired *t* test). Therefore, there was no significant increase in collateral flow that could account for the protective effect of PGI_2 on the evolving infarct mass. Endocardial/epicardial blood flow ratios were calculated to determine if the blood was being redistributed within the myocardium. Endocardial/epicardial flow ratios remained unchanged with treatment (control pretreatment 1.38 ± 0.07 ml/min/gm, after vehicle 1.38 ± 0.08 ml/min/gm; PGI_2 pretreatment 1.17 ± 0.03 ml/min/gm, after PGI_2 1.08 ± 0.09 ml/min/gm) in the region of the myocardium perfused by the LCCA. The endocardial/epicardial ratios during occlusion within the ischemic area were 0.4 ± 0.11 for the PGI_2 group and 0.15 ± 0.40 for the control group ($p > 0.05$). This again confirms that the protective effect of PGI_2 on infarct size is independent of blood flow redistribution. Blood flows in the area of the heart not rendered ischemic are depicted on the *right side* of Fig. 3. As is evident from the figure, there were no significant differences in blood flows in this region with time (paired *t* test) or between groups (control, PGI_2) at any time tested (unpaired *t* test). Endocardial/epicardial flow ratios in the nonischemic regions were similar to endocardial/epicardial ratios obtained in the region perfused by the LCCA.

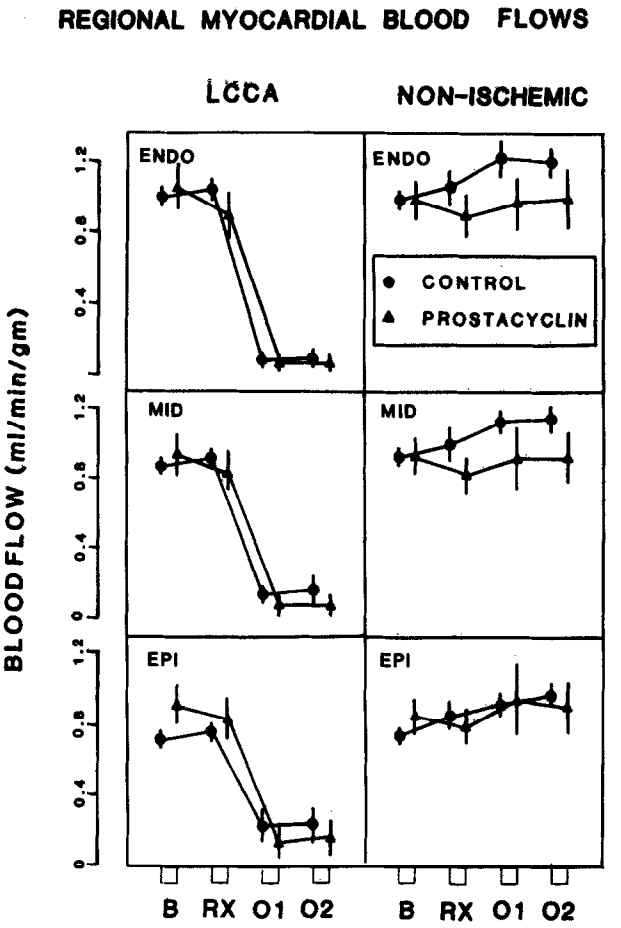

Fig. 3. Regional myocardial blood flow to the region of the heart that is rendered ischemic is depicted on the *left half* of the figure. On the *right* is the nonischemic region. Subendocardial *(ENDO)* blood flow is depicted in the *top two panels*, midmyocardial *(MID)* flow is shown in the *middle two panels*, and subepicardial *(EPI)* flow is illustrated in the *bottom two panels*. Baseline *(B)* blood flow represents before PGI_2 or vehicle infusion. *RX* represents 25 minutes after PGI_2 or vehicle infusion. *01* and *02* represent 10 and 80 minutes, respectively, after LCCA occlusion. There were no significant differences between groups (Student's *t* test) in any region or at any time point (Control = *filled circles*; PGI_2 = *filled triangles*).

Histology. Representative transmural sections from each of 21 hearts were used to assess relative degree of ischemic injury by light microscopy. Tissue samples were prepared for histologic examination such that regions from the central infarct area, the border region between normal tissue and necrotic tissue, and the normal tissue were analyzed. No significant differences were observed among the three treatment groups (control, PGI_2, SC39902) with respect to the appearance of the necrotic tissue in the central infarct area. No differences among treatment groups were discernible with respect to the extent of hemorrhage in the infarct area or

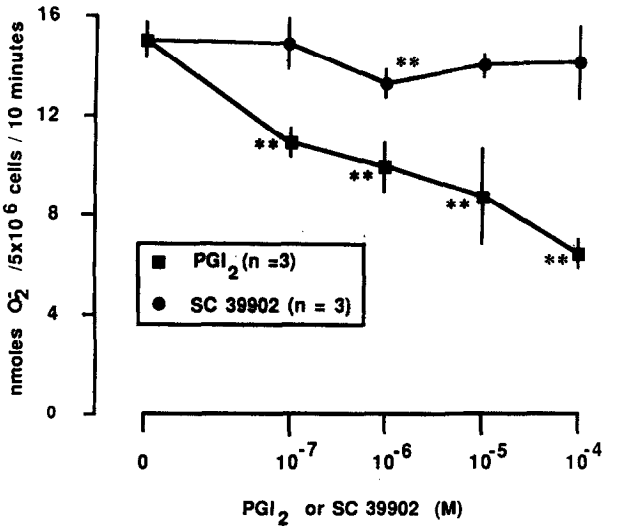

Fig. 4. Inhibition of superoxide generation by PGI_2 but not by SC39902. Canine neutrophils were activated with opsonized zymosan. PGI_2 produced a concentration-dependent inhibition of O_2^- generation while SC39902 weakly inhibited only at 10^{-6}M. Each symbol represents the mean ± S.E.M. of three determinations except that n = 6 for 0 PGI_2 or SC39902. PGI_2 or SC39902 were serially diluted in cold Hank's buffer immediately before addition to the tubes. $^{**}p < 0.001$ compared to O_2^- in the absence of PGI_2 or SC39902.

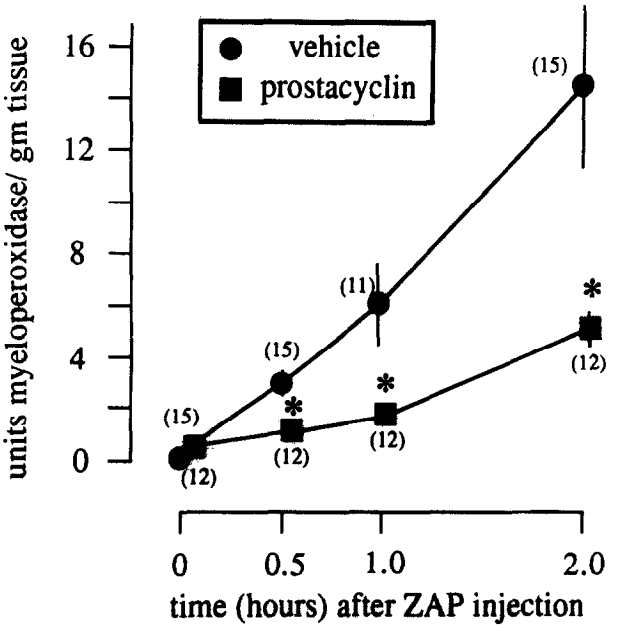

Fig. 5. Inhibition of neutrophil migration into inflammatory skin lesions. The number of individual biopsies is in *parentheses. Vertical lines* represent the standard error of the mean ($^{**}p < 0.01$, Student's *t* test). The symbols represent the mean of four vehicle treated and 3 PGI_2 treated dogs. PGI_2 dose was 50 ng/kg/min intravenously.

border regions. Gross inspection of histologic sections revealed no discernible differences in relative neutrophil content in infarcted tissue or border regions.

Frequency of ventricular fibrillation. The development of spontaneous ventricular fibrillation (VF) was monitored in this study to determine whether PGI_2 or SC39902 may affect the frequency of VF. Previous reports suggest that PGI_2 and PGI_2 analogues may be either antiarrhythmic or arrhythmogenic, depending on the dose of drug used.[26,27] The overall incidence of VF was 7 of 48 dogs in the study with control frequency of 4 of 19, for PGI_2 it was 2 of 18 dogs, and for SC39902 it was 1 of 11 dogs (chi square = 1.166, $p > 0.05$). Therefore was no difference in the relative frequency of observed in this study.

In vitro superoxide production by canine neutrophils. Canine neutrophils were stimulated with OZ and superoxide O_2^- generation was monitored spectrophotometrically (at 550 mn) by the superoxide dismutase inhibitable reduction of ferricytochrome C. Fig. 4 demonstrates that prostacyclin produces a concentration-dependent inhibition of neutrophil activation. The rate of superoxide production in the absence of PGI_2 or SC39902 was 15.23 ± 0.22 nmoles $O_2^-/5 \times 10^6$ cells/10 minutes. PGI_2 at a concentration of 10^{-7}M inhibited O_2^- production by 27% compared to control (no PG). At a PGI_2 concentration of 10^{-4}M, superoxide production was inhibited by 55% compared to control. In contrast, SC39902 did not show a concentration-dependent inhibition of superoxide production. Moreover, SC39902 at a concentration of 10^{-6}M was the only concentration that was found to be significantly different from control. At this concentration, SC39902 inhibition was only 11% compared to control.

Neutrophil migration into dermal inflammatory lesions. Fig. 5 shows that prostacyclin at 50 ng/kg/min effectively inhibits neutrophil migration into skin lesions induced by activated complement fragments (primarily C5a desArg). The myeloperoxidase content at the time of zymosan-activated plasma (ZAP) injection was 0.41 ± 0.08 U/gm in the vehicle treated group and 0.54 ± 0.24 U/gm in the PGI_2 treated group ($p > 0.05$). The myeloperoxidase content of skin lesions was significantly less at 0.5, 1.0 and 2.0 hours after ZAP injection (all $p < 0.01$ compared to control). Furthermore, neutrophilic infiltration in the PGI_2 treated dogs was only 36.3% of the control value at the 2 hour time point (14.68 ± 3.01 U/gm vs 5.33 ± 0.65 U/gm).

DISCUSSION

A number of investigators have attempted to define the relevant mechanism for the myocardial protection by prostacyclin in the setting of experimental myocardial ischemia.[6-10, 28, 29] The reduction of arterial blood pressure by PGI_2 would at least theoretically reduce myocardial ischemia by reducing afterload and thus myocardial oxygen demand.[30, 31] In the present study, myocardial oxygen consumption as estimated by the rate-pressure product[25] was reduced to the same extent by PGI_2 and SC39902 (Table I). Prostacyclin resulted in a 59% reduction in infarct size with respect to the area at risk compared to controls, whereas SC39902 failed to reduce infarct size (Fig. 2). The relevant mechanism of protection by PGI_2 is therefore not due to a reduction in myocardial oxygen demand.

Jugdutt et al.[6] demonstrated that PGI_2, by virtue of its vasodilatory properties, increased collateral blood flow to the ischemic myocardium in closed-chest conscious dogs subjected to permanent coronary artery occlusion. Indeed, in the aforementioned study, protection of the ischemic myocardium after permanent coronary artery occlusion is probably dependent upon the ability of PGI_2 to increase collateral blood flow, since PGE_1 and PGI_2 reduced infarct size while PGE_2 did not. Prostaglandins E_1 and I_2 increased collateral blood flow in that study but PGE_2 did not, while all three prostaglandins produced a similar reduction in blood pressure. In another study, Melin and Becker[10] demonstrated that even in the absence of increased collateral blood flow to the ischemic myocardium, PGI_2 treatment resulted in a reduction in myocardial necrosis. We report that PGI_2 reduced infarct mass without increasing collateral blood flow to the ischemic myocardium during 90 minutes of regional ischemia. LCCA blood flow during reperfusion was similar among the three treatment groups (control, PGI_2, SC39902; Fig. 1), suggesting that the mechanism of protection was independent of changes in blood flow to the previously ischemic myocardium and was not related to the "no-reflow" phenomenon.[32]

We hereby offer an alternative explanation for the cytoprotective effect of PGI_2 in ischemic myocardium in vivo. Prostacyclin can inhibit human neutrophil stimulation by formylmethionyl-leucyl-phenylalanine (f-Met-Leu-Phe) and zymosan activated serum (complement activation products) as determined by superoxide production and lysosomal enzyme release in vitro.[14-16] In addition, prostaglandins of the E series inhibit neutrophil chemotaxis, superoxide production, and degranulation in a variety of species, presumably through an increase in neutrophil intracellular concentration of cyclic adenosine monophosphate (AMP).[16] We report in this study that PGI_2 can effectively inhibit canine neutrophil activation in vitro as measured by superoxide-specific reduction of ferricytochrome C. The PGI_2 analogue SC39902 manifests hemodynamic effects similar to those of PGI_2 in vivo (i.e., reduces blood pressure) and also inhibits platelet aggregation in vitro (unpublished observations). A recent report[33] demonstrated that SC39902 is an effective inhibitor of platelet activation in vivo as measured by the ability to prevent adenosine diphosphate (ADP)-induced thrombocytopenia in rats. Despite these similarities to PGI_2, the analogue SC39902 failed to produce effective inhibition of canine neutrophil activation in vitro when stimulated with OZ. Furthermore, SC39902 does not protect the ischemic-reperfused myocardium with respect to ultimate infarct size. We propose, therefore, that the relevant mechanism for PGI_2 protection in our experimental model is via the inhibition of neutrophil activation.

The concentration of PGI_2 that produces 50% inhibition of neutrophil O_2^- production is very similar to the 30 μM of PGI_2 for 50% inhibition reported by Fantone and Kinnes.[14] The concentrations of PGI_2 achieved in vivo with an infusion rate of 50 ng/kg/min would be expected to approach the concentration range of 10^{-7} in vivo. Furthermore, inhibition of neutrophil migration in vivo by PGI_2 (50 ng/kg/min) was demonstrated and is likely to be the relevant mechanism of protection of the ischemic reperfused myocardium.

The proinflammatory role of the neutrophil during myocardial ischemia and reperfusion injury has been demonstrated.[11] Hill and Ward[34] in 1971 demonstrated that complement is activated via the alternative pathway during myocardial ischemia. Maroko et al.[35] depleted dogs of complement component 3 (C3) with cobra venom factor. Complement depletion resulted in reduced tissue damage and reduced neutrophil infiltration into the ischemic myocardium. Pinckard et al.[36] extended these observations to the baboon, and demonstrated complement localization within the ischemic myocardium by direct immunofluorescence methods. Neutrophils, when activated by complement fragments, produce oxygen-derived free radicals and release degradative lysosomal enzymes, both of which are capable of mediating tissue destruction.

Using another approach, Romson et al.[12] demonstrated a 44% reduction in the mass of myocardial infarct that evolved when dogs were made neutropenic before the induction of regional myocardial ischemia. In a related study, Romson et al.[13] demonstrated that the protective effect of ibuprofen (a nonsteroidal anti-inflammatory agent) upon the ischemic/reperfused myocardium was correlated with the ability of the drug

to inhibit the infiltration of neutrophils into the ischemic myocardium. Kuehl et al.[37] demonstrated that PGI_2 inhibits neutrophil lipoxygenase activity. Neutrophil lipoxygenase products provide an important proinflammatory amplification signal. Leukotriene B_4, produced by neutrophils, is a potent chemoattractant for neutrophils, macrophages, and blood monocytes. Inhibition of lipoxygenase by PGI_2 would therefore result in a reduction in phagocytic cell infiltration and thus reduce cellular necrosis.

In summary, we report the effectiveness of PGI_2 for reducing myocardial infarct mass in the dog with temporary regional myocardial ischemia and reperfusion. The PGI_2 analogue SC39902 produced a similar reduction in arterial blood pressure and heart rate but failed to reduce infarct mass. In vitro canine neutrophil activation was inhibited in a concentration-dependent manner by PGI_2, whereas SC39902 failed to effectively inhibit neutrophil activation over the same concentration range. The data on neutrophil migration into inflammatory skin lesions demonstrate that neutrophil function is affected in vivo at the same dose of PGI_2 that reduces myocardial ischemic reperfusion injury. These data suggest that the cytoprotective effect of PGI_2 in this experimental model of myocardial infarction is due to inhibition of neutrophil activation.

The studies described in this report may have relevance with respect to the clinical management of patients who are evolving an acute myocardial infarction and who become candidates for thrombolytic therapy with streptokinase or perhaps tissue plasminogen activator. There are sufficient data to suggest that PGI_2 is capable of preventing platelet aggregation[2] and that the prostanoid may be of value as an adjunct to thrombolytic therapy, whereby it may serve to prevent reocclusion of the successfully recanalized coronary artery.[38] The data presented in this report might give added importance for the concomitant use of PGI_2 along with thrombolytic therapy as an approach for the prevention of that component of myocardial injury associated with reperfusion of the ischemic heart. Furthermore, our data with respect to the cytoprotective effect of PGI_2, while in agreement with that of previous investigators,[5-10] provide an explanation for the mechanism by which PGI_2 elicits its beneficial action through an inhibition of neutrophil function.

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Hemodynamic effects of BTS 49465, a new long-acting systemic vasodilator drug, in patients with severe congestive heart failure

The hemodynamic effects of BTS 49465, a new oral, direct-acting systemic vasodilator drug, were investigated in 10 patients with severe chronic congestive heart failure. One to 2 hours after the administration of 1.5 mg/kg orally, BTS 49465 produced significant increases in cardiac index, stroke volume index, and stroke work index (26%, 27%, and 23%, respectively, $p < 0.01$ to 0.001) and marked decreases in left ventricular filling pressure (−12.6 mm Hg, 44%), mean pulmonary artery pressure (−13.2 mm Hg, 31%), and mean right atrial pressure (−7.7 mm Hg, 63%), all $p < 0.001$, without significant changes in heart rate. These hemodynamic responses were accompanied by notable declines in systemic vascular resistance (−28%, $p < 0.001$) and pulmonary arteriolar resistance (−24%, $p < 0.05$). These effects persisted throughout the 24-hour period of observation. The decline in left ventricular filling pressure in our patients ranged in magnitude from 8 to 21 mm Hg, and varied linearly and directly with pretreatment values for left ventricular filling pressure ($r = 0.69$). The decrease in systemic vascular resistance ranged in magnitude from 3% to 40% and varied linearly and directly with pretreatment values for systemic vascular resistance ($r = 0.85$). These data indicate that BTS 49465, a new oral, direct-acting vasodilator agent, exerts balanced cardiocirculatory effects in patients with severe chronic heart failure, which may be sustained with once-daily oral administration. (Am Heart J 1987;113:137.)

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