Endothelium and Myocardial Protecting
Actions of Taprostene, a Stable Prostacyclin
Analogue, After Acute Myocardial Ischemia
and Reperfusion in Cats

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The effects of taprostene, a synthetic prostacyclin analogue, were investigated in a 6-hour model of myocardial ischemia (MI) with reperfusion in anesthetized cats. Taprostene (100 ng/kg/min) was infused intravenously starting 30 minutes postocclusion of the left anterior descending coronary artery followed by reperfusion 1 hour later, and the cats were observed for an additional 4.5 hours. Taprostene infusion resulted in significantly lower plasma creatine phosphokinase activities at every time from 3 to 6 hours for the MI+taprostene group compared with the MI+vehicle group and were not significantly different when compared with sham MI controls. The areas at risk, expressed as a percentage of the total left ventricular weights, were not significantly different between the MI groups. However, the necrotic area expressed as a percentage of the myocardial area at risk was significantly lower in the taprostene-treated cats compared with the untreated MI group (p<0.01). Cardiac myeloperoxidase activities indicated that significantly fewer neutrophils were attracted to the area at risk and to the ischemic zone of the MI+taprostene cats when compared with the MI cats given only the vehicle. Data from isolated left anterior descending coronary artery ring preparations removed from hearts after 6 hours of ischemia indicated that the endothelium was damaged by ischemia-reperfusion injury in the untreated cats. However, endothelial dysfunction was not observed in circumflex coronary arteries of ischemic cats or in coronary rings isolated from MI+taprostene cats. Thus, taprostene exerted a significant cardioprotection in cats subjected to ischemia and reperfusion. This cardioprotection appears to be related to protection of coronary endothelium integrity and prevention of neutrophil accumulation in the ischemic myocardium. (Circulation Research 1990;66:1362–1370)

Prostacyclin (PGI₂) is a major product of the metabolism of arachidonic acid produced by the vascular endothelium. PGI₂ has a profile of cardiovascular effects that include systemic hypotension and coronary vasodilation. In addition to these hemodynamic effects, PGI₂ also has cytoprotective actions such as antiaggregatory actions on platelets, and stabilization of erythrocyte and lysosomal membranes. As a result of this profile of effects, PGI₂ has been shown to be beneficial in a variety of models of ischemia and shock. In particular, PGI₂ has been shown to exert protection in myocardial ischemia. However, PGI₂ has a very short half-life at physiological pH in aqueous media (i.e., about 3 minutes) due to chemical instability and rapid metabolic degradation.

Iloprost is a stable analogue of PGI₂ that was found to have potential therapeutic utility in ischemic and circulatory shock states. Iloprost has a profile of actions similar to that of PGI₂ and has been shown to exert protective effects in myocardial ischemia and in traumatic shock. However, PGI₂ and iloprost are potent vasodilators that may account for some of the unwanted side effects (i.e., anxiety and headache) of PGI₂ when tested in clinical trials.

Therefore, new stable analogues of PGI₂ have been developed that optimize cytoprotective effects while diminishing vasodepressor actions coupled with longer half-lives due to increased chemical stability. Taprostene (CG-4203) ([5Z,13E,9,11,15S]-2,3,4-trinor-1,5-inter-m-phenylene-6,9-epoxy-11,15-dihydroxy-
obtain an infusion rate that produced minimal hemodynamic (i.e., vasodilator) effects but still exerted cardioprotective effects. The optimal rate of taprostene infusion was determined to be 100 ng/kg/min. Cats infused with the vehicle received 1.2 ml/hr of 0.9% NaCl over the same time period. Four experimental groups were studied: sham myocardial ischemia (MI)+vehicle (n=4), sham MI+taprostene (n=7), MI+vehicle (n=6), and MI+taprostene (n=7). The sham MI cats were subjected to all procedures except that the ligature around the LAD was not tightened.

Blood Sampling

Arterial blood samples (2 ml) were drawn immediately before ligation and hourly thereafter. The blood samples were collected in tubes containing 200 IU heparin sodium. Samples were centrifuged at 2,500g and 4°C for 20 minutes. The plasma was drawn off and analyzed spectrophotometrically for creatine kinase (CK) activity according to the method of Rosalki,21 and for protein concentration by the biuret method.22 Plasma CK specific activity was expressed as international units per milligram protein times 1,000.

Myocardial Tissue Analysis

At the end of the 6-hour experimental period, the ligature around the LAD was retightened to completely occlude the vessel. At that time, 30 ml of 0.5% Evans blue was injected into the left atrium to stain that area of the myocardium perfused by the patent coronary arteries. The area at risk was thus determined by negative staining. The heart was rapidly excised, and the right ventricle and great vessels were removed. The left ventricle was then sliced parallel to the atrioventricular groove in 3-mm-thick sections. The unstained portion of the myocardium (i.e., the total area at risk) was separated from the rest of the myocardium (i.e., the area not at risk). That portion of the myocardium at risk was again sliced into thin slices (i.e., 1 mm thick) and incubated in 0.1% solution of nitro blue tetrazolium in phosphate buffer at pH 7.4 and 37°C for 15 minutes. The tetrazolium dye forms a blue formazan complex in the presence of coenzymes and dehydrogenase.23 The injured or necrotic portion of the myocardium at risk that did not stain was separated from the stained portion of the myocardium (i.e., the ischemic but nonnecrotic area). All three portions of left ventricular myocardium (i.e., nonschematic, ischemic nonnecrotic, and ischemic necrotic) were weighed and stored at −70°C for subsequent assay of myeloperoxidase (MPO) activity.

Determination of Tissue Myeloperoxidase Activity

Cardiac MPO activity, a specific enzyme constituent of neutrophils, was determined in myocardial tissue by the method of Bradley et al24 as modified by Mullane et al.25 MPO activity represents a direct correlation of neutrophil content, which accumulates in the presence of myocardial injury. Cardiac tissue was stored at −70°C until homogenization. To mea-
sure MPO activity, the myocardium was homogenized in 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical, St. Louis, Missouri) dissolved in 50 mM potassium phosphate buffer at pH 6 by using a Polytron (PCU-2, Kinemutica GmbH, Lucerne, Switzerland) homogenizer for 15 seconds×2 at a setting of 7,000 rpm. Homogenates were centrifuged for 20 minutes at 12,500g at 4°C. The supernatants were then collected and reacted with 0.167 mg/ml o-dianisidine dihydrochloride (Sigma Chemical) and 0.0005% H2O2 in 50 mM phosphate buffer at a pH of 6. The change in absorbance was measured spectrophotometrically at 460 nm. One unit of MPO activity is defined as that quantity of enzyme degrading 1 μmol H2O2/min at 25°C.

**Artery Ring Preparation**

Segments of both the LAD and the left circumflex coronary artery (LCX) were excised at the end of the experiment just after Evans blue staining, which was used to demarcate the area at risk from the area not at risk. These segments were then dissected free of connective tissue, cut transversely into rings, and connected to force transducers in 20-ml chambers filled with Krebs-Henseleit solution of the following millimolar composition: NaCl 120, glucose 10, NaHCO3 125, KCl 4.74, KH2SO4 1.19, EDTA 0.016, and CaCl2 2.5. The Krebs-Henseleit solution was bubbled with 95% O2-5% CO2 and temperature was maintained at a constant 37°C by means of a circulating water bath surrounding the chamber. Resting force was set at 0.5 g, and the rings were allowed to equilibrate for 90–120 minutes. This resting force was that force on the small artery rings at which a maximal response to U46619 could be obtained. At that time, the rings were precontracted with 30 nM U46619 (9,11-methanoepoxy-prostaglandin H2. The Upjohn Company, Kalamazoo, Michigan), a thromboxane mimic. The rings were then subjected to sequential additions of acetylcholine (ACh) 0.01–10 nM and acidified sodium nitrite (NaNO2, pH 2.0) 0.1–100 μM. ACh produces endothelium-dependent smooth muscle relaxation, whereas acidified NaNO2 produces direct smooth muscle relaxation and thus is not endothelium dependent. Additional experiments were performed in cat thoracic aortic rings precontracted with 2 μM norepinephrine, and then taprostone at 1 ng/ml to 1 μg/ml was tested for its ability to relax the aortic rings.

**Data Analysis**

All values in the text and figures are presented as mean±SEM of n independent experiments. The data were analyzed by using analysis of variance with repeated measures when appropriate. Differences between specific means were tested by post hoc analysis using Student’s t test with the Bonferroni correction for multiple comparisons. Values of p<0.05 were considered to be statistically significant.

![Figure 1](http://circres.ahajournals.org/)  
**Figure 1.** Pressure-rate index expressed as mm Hg×(beats/min)/1000 sampled hourly during the 6-hour myocardial ischemia (MI) followed by reperfusion. All values are mean±SEM; n=number of cats in each group; sham MI+taprostene group, n=6; MI+vehicle group, n=6; MI+taprostene group, n=7. MABP, mean arterial blood pressure; HR, heart rate; D, drug; V, vehicle.

**Results**

To assess the severity of the ischemia soon after LAD occlusion, ST segment elevations were monitored every 20 minutes in all groups. Peak ST segment elevations occurred 20–40 minutes postocclusion and were not significantly different between the two MI groups (MI+vehicle, 0.13±0.4 mV, n=6; MI+taprostene, 0.14±0.5 mV, n=7). Furthermore, peak ST segment elevations in both MI groups were significantly different from the sham MI+vehicle and the sham MI+taprostene groups (p<0.01). Therefore, both MI groups were subjected to a comparable degree of ischemic insult as a result of the LAD occlusion.

Figure 1 illustrates the time course of the pressure-rate index used as an index of myocardial oxygen demand in all groups of cats studied. The pressure-rate index of all the groups was not significantly different from each other at time zero. At 1 hour, the pressure-rate indexes of all groups had fallen after occlusion or sham occlusion of the LAD. From 2 hours onward, the pressure-rate indexes of all groups remained relatively stable with no significant differences among the groups at any time. Moreover, no significant differences were observed in heart rates or mean arterial blood pressures, indicating that none of the interventions used reduced myocardial oxygen demand, and thus any cardioprotective effect of taprostene cannot be attributed to a reduction in afterload or myocardial oxygen demand. Moreover, in sham MI+vehicle cats, the pressure-rate index decreased from 24.1±1.1 to 20.8±1.5, a decrease of 3.3, compared with a decrease of 3.6 in the sham
MI+taprostene group, this difference not being significant. Thus, taprostene did not exert a significant effect on systemic hemodynamics in cats at the infusion rate used in this study.

Figure 2 summarizes the changes in plasma CK activity in all groups of cats studied. Modest increases in plasma CK activity were seen in the sham MI group throughout the 6-hour observation period. In sham MI+vehicle cats, plasma CK activity increased 10.7±1.9 units/mg protein x 10^-3 in comparison with 13.1±3.5 units/mg protein x 10^-3 in the sham MI+taprostene group. These values are not significantly different from each other. However, large increases in plasma activity of CK were observed in MI+vehicle cats. The MI+taprostene group experienced a marked attenuation of plasma CK activity such that these values did not differ significantly from the plasma CK activities observed in the sham MI group. The plasma CK activities of the MI+taprostene group, as well as the sham MI group, were both significantly different than the MI+vehicle group at each hour beyond 3 hours. Thus, treatment with taprostene effectively inhibited plasma accumulation of CK activity, an indication that taprostene exerted a salutary effect in ischemia and reperfusion.

In addition to the plasma CK activity, we used an anatomic estimation of the amount of resulting necrotic tissue as another index of ischemic injury. The wet weights of the areas of myocardium subjected to ischemia (areas at risk) expressed as a percentage of the total left ventricular weights were not significantly different between either of the MI groups, as shown in Figure 3, indicating that the region of myocardium subjected to ischemia was comparable in both MI groups. Neither sham MI group exhibited any detectable necrotic area. The weight of the necrotic myocardial tissue expressed as a percentage of the weight of the myocardial area at risk was 18±3% in the MI+vehicle group. In contrast, the MI+taprostene group had a smaller percentage (3±1%) of necrotic myocardial tissue that was significantly less than the MI+vehicle group (p<0.01). This difference existed whether the necrotic area was expressed as a percentage of area at risk or as a percentage of the total left ventricle (see Figure 3). Thus, the taprostene treatment exerted a significant reduction in ischemic damage in the cat after ischemia and reperfusion.

One of the mechanisms thought to be responsible for reperfusion injury is adherence of neutrophils to the vascular endothelium of the ischemic region. MPO activity is specific for neutrophils and can therefore be used as a marker for neutrophil accumulation in the heart. Figure 4 summarizes these results. Very low cardiac MPO activities were observed in the sham MI group. In both sham MI groups, there was no detectable MPO activity in the necrotic area. In the area at risk, the MPO activity was 0.12±0.08 units/100 mg tissue in the sham MI+vehicle group and 0.06±0.02 units/100 ng tissue in the sham MI+taprostene group. These values are not statistically different from each other. However, marked increases in MPO activity were observed in the untreated MI group in the area at risk and in the necrotic zone to values which were significantly above those of the sham MI group. The MI group given taprostene exhibited significantly lower MPO activi-
ties in both the area at risk \((p<0.05)\) and the necrotic area \((p<0.01)\) when compared with the MI+vehicle group. Moreover, there were no significant differences among the groups in MPO activity in the area of the myocardium that was not at risk. Therefore, taprostene treatment appeared to inhibit neutrophils adhering to the myocardial endothelium in both jeopardized and necrotic myocardial tissue after ischemia and reperfusion.

Figure 5 illustrates typical recordings of developed force obtained from cat coronary arterial ring preparations. These data were obtained from coronary rings isolated from a cat subjected to myocardial ischemia and reperfusion and administered only the vehicle (i.e., 0.9% NaCl). The upper pair of tracings illustrates the response of coronary rings taken from the left anterior descending branch of the left coronary artery, which was subjected to ischemia and reperfusion. The lower pair of tracings illustrates the response of coronary rings taken from the LCX branch, which was not subjected to ischemia and reperfusion. Recordings on the left side of the figure illustrate the response to ACh, which is an endothelium-dependent vasodilator. Recordings on the right side of the figure illustrate the response to acidified NaNO2, which is an endothelium-independent vasodilator. The upper left recording clearly shows an impaired ability to respond to ACh in the LAD rings, while the tracing in the upper right shows that the endothelium-independent vasodilator, acidified NaNO2, is capable of producing a full vasodilation in the same ring. These data indicate that a functional deficit exists in the endothelium because the vascular smooth muscle responds normally to both the vasoconstrictor U46619 and the endothelium-independent dilator, acidified NaNO2. The lower pair of recordings illustrates normal relaxation in response to both ACh and acidified NaNO2 in the LCX rings, which were used as a control.

Figure 6 summarizes the vasorelaxant responses to ACh in cat coronary artery rings taken from the LAD (left panel) and from the LCX (right panel) in all three groups studied. In the left panel, LAD rings from sham MI+taprostene cats responded to ACh...
artery rings to relax completely (i.e., not significantly different from 100%) in response to acidified NaNO₂.

The panel on the right illustrates the percentage of relaxation produced in response to acidified NaNO₂ in coronary artery rings taken from the LCX. There were no significant differences among any of the groups in the ability of these coronary artery rings to relax completely in response to acidified NaNO₂. These results indicate a normal vasorelaxant ability of coronary vascular smooth muscle in both the experimental and control coronary arteries to respond to direct (i.e., non-endothelium-dependent) dilators.

**Discussion**

The data presented in this study clearly show that taprostene exerts significant cardioprotective effects in the cat myocardium subjected to ischemia and reperfusion. LAD occlusion for 1.5 hours resulted in an ST segment elevation, indicating a significant insult to ischemic cardiac tissue. Other signs of ischemia were the dark, cyanotic color of the epicardium distal to the ligation and subsequent bulging in this area of the left ventricle.

We obtained several lines of evidence including anatomic, biochemical, and physiological data that taprostene exerts a cardioprotective effect in ischemia and reperfusion. First, plasma CK activities, indicative of the severity of ischemic injury to the myocardium, were significantly lower in cats treated with taprostene than those cats receiving only the vehicle. The degree of protection observed in the MI+taprostene group was so effective that plasma CK activities were not significantly different from sham MI cats at any time during the 6-hour observation period. Thus, taprostene prevented the loss of CK from ischemic tissue during the first 6 hours after myocardial ischemia, suggesting a significant cardioprotective effect.

Second, analysis of myocardial tissue clearly indicated that the ischemia groups were exposed to a comparable degree of myocardial jeopardy as a result of LAD occlusion. However, a relatively large proportion of the area at risk became necrotic myocardial tissue in untreated MI cats. Taprostene prevented much of this mass of ischemic tissue from becoming necrotic, whether calculated as a percentage of the mass at risk or as a percentage of the total left ventricular mass. These findings suggest that treatment with taprostene preserves the ischemic myocardium and prevents tissue necrosis during the first 6 hours of ischemia. Whether this protection is longer lasting is not known at present.

Third, markedly lower MPO activity was observed in ischemic hearts treated with taprostene than in untreated ischemic hearts. This beneficial effect of taprostene suggests an inhibition of adherence of neutrophils to the endothelium. Taprostene may exert a direct protective effect on endothelial cells or it may prevent neutrophils from releasing oxygen free radicals, catabolic enzymes, and other deleterious substances (e.g., leukotrienes, cytokines) during isch-
emic conditions or after reperfusion.\textsuperscript{26,27} This may be particularly relevant to endothelial function because superoxide radicals are known to be one of the most important naturally occurring inactivators of endothelium-derived relaxing factor, thought to be nitric oxide.\textsuperscript{28} Thus, reduced MPO activity, presumably caused by reduced neutrophil accumulation in the ischemic myocardium, may be a key aspect of the cytoprotective effect of taprostene.

Fourth, the coronary ring data indicate that ischemia and reperfusion produce endothelial damage characterized by the marked inability of rings from ischemic hearts to fully relax when presented with an endothelium-dependent vasodilator. In contrast, vascular rings taken from MI+taprostene cats demonstrated a normal degree of relaxation. Thus, endothelial injury\textsuperscript{29,30} may be an important component of reperfusion injury, which is prevented by taprostene infusion.

There are a variety of possible mechanisms by which taprostene could provide cardioprotection including 1) hemodynamic effects such as a direct vasodilator effect on vascular smooth muscle and a reduced cardiac afterload, 2) cytoprotection of vascular endothelial cells, 3) prevention of activation of neutrophils thus inhibiting their release of mediators, and 4) a combination of several of these mechanisms.

Both PG\textsubscript{I\alpha} and taprostene have been shown to have a direct vasodilator effect on vascular smooth muscle by activation of adenylate cyclase, leading to the production of cyclic AMP, thus inhibiting the contractile process and inducing relaxation.\textsuperscript{2,15} However, taprostene has been designed specifically to minimize vasodilation at concentrations that produce cytoprotective effects. In six cat aortic rings, taprostene at concentrations of 1–100 ng/ml failed to exert any vasorelaxant effect. At 300 ng/ml, taprostene relaxed the vascular rings 34±3%. The EC\textsubscript{50} for taprostene in this preparation was calculated to be 520 ng/ml, a value 26 times that of its antiplatelet aggregatory effect in cat platelet-rich plasma.\textsuperscript{15} In this study, the dose of taprostene used was specifically chosen to avoid systemic vasodilation, and indeed, no evidence of systemic vasodilation was observed. Therefore, taprostene probably does not protect the ischemic myocardium by reducing myocardial oxygen demand.

Ischemia and reperfusion lead to release of vasoactive mediators that may be involved in functional alterations and damage to the endothelium. Among these are products of cyclooxygenase and lipoxygenase metabolism of arachidonic acid. These products and others have been shown to aggregate platelets, constrict coronary vascular smooth muscle (e.g., thromboxane A\textsubscript{2}, leukotriene D\textsubscript{4}),\textsuperscript{31–33} and recruit neutrophils (e.g., leukotriene B\textsubscript{4}). Damage or loss of endothelial cells would allow circulating mediators direct access to the underlying smooth muscle cells where they could produce effects on vascular tone or inhibit smooth muscle cell function. Data from the coronary artery ring preparations clearly show that taprostene contributes to a functional preservation of the endothelium in terms of its ability to respond to endothelium-dependent vasodilators.

Taprostene exerts antiplatelet activity in a variety of species\textsuperscript{15,34,35} and also has platelet disaggregatory activity.\textsuperscript{2} The endothelium normally provides a nonthrombogenic surface that prevents platelets from aggregating and adhering when the coagulation cascade is activated, thus protecting the underlying myocytes. The interactions of taprostene with other vasoactive substances has not yet been fully explored. However, it has been demonstrated that nitric oxide, the most probable identity of endothelium-derived relaxing factor and PG\textsubscript{I\alpha} have synergistic effects in the inhibition of platelet aggregation. Endothelial cells are known to produce endothelium-derived relaxing factor and PG\textsubscript{I\alpha}, in response to thrombin and other products released by aggregating platelets. It is evident from the data on coronary artery rings that taprostene protected against endothelial damage produced acutely in myocardial ischemia and reperfusion, thus maintaining an intact thromboresistant surface.

Neutrophils and products of neutrophils have been associated with a component of damage induced in myocardial ischemia and reperfusion.\textsuperscript{36,37} It is known that the complement system is activated in ischemic tissue,\textsuperscript{38} producing factors that cause neutrophils to migrate along chemotactic gradients to areas of injury and damage, adhere to endothelial cells, and ultimately infiltrate damaged and necrotic areas. Significant reductions in myocardial damage have been accomplished after experimental coronary occlusion by inactivation of the complement system.\textsuperscript{39} Moreover, oxygen-derived free radicals produced by neutrophils\textsuperscript{36,40,41} are also thought to play a role in myocardial cell injury. We have shown that taprostene prevents the accumulation and adherence of neutrophils both in the area at risk and in the necrotic zone, thus limiting the opportunities for microvascular plugging by neutrophil aggregates and reducing the opportunity for neutrophil-induced damage by toxic product release. These effects occurred despite any effect of taprostene on the actual size of the area at risk. In this regard, cytokines (e.g., tumor necrosis factor and interleukin-1\beta) released from neutrophils have recently been found to induce endothelial cell expression of a neutrophil chemotactic factor.\textsuperscript{42} Thus, there is an intimate interrelationship between activated neutrophils and endothelial cells, which taprostene may inhibit. Further investigation is necessary to clarify these interrelationships.

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