

## BENEFICIAL EFFECTS OF ILOPROST ON ACUTE MYOCARDIAL ISCHEMIA IN DOGS

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### ABSTRACT

The effects of intravenous administration of iloprost, a prostacyclin analogue, on myocardial energy metabolism and myocardial blood flow (MBF) were examined in anesthetized open-chest dogs subjected to 60 min of myocardial ischemia by coronary ligation. Iloprost administration at levels of 0.1 or 0.2  $\mu\text{g}/\text{kg}/\text{min}$  was started 30 min before the commencement of ischemia and continued throughout the 90 min observation period. Since systolic aortic pressure in the iloprost 0.2  $\mu\text{g}/\text{kg}/\text{min}$  group showed significantly lower values than that in the control group, whereas no clear effect was observed with the lower concentration (0.1  $\mu\text{g}/\text{kg}/\text{min}$ ), this latter group was further investigated. This 0.1  $\mu\text{g}/\text{kg}/\text{min}$  dose of iloprost lacked influence on MBF in both ischemic and nonischemic areas but did result in a significantly higher value for high energy phosphate contents in the ischemic myocardium. Moreover, myocardial mitochondrial respiratory function in the ischemic area was significantly improved. These results indicate that iloprost brought about preservation of myocardial energy metabolism without alteration of coronary perfusion, suggesting that it may exert a direct cardioprotective effect.

Key Words: Iloprost, Canine heart, Ischemia, Myocardial blood flow, Myocardial energy metabolism

### INTRODUCTION

Iloprost is well recognized as a prostacyclin analogue that has similar pharmacologic action but is chemically more stable.<sup>1)</sup> Several studies have documented beneficial effects of this agent on ischemic myocardium,<sup>2-5)</sup> as well as its platelet antiaggregating<sup>6-8)</sup> and cytoprotective action.<sup>9,10)</sup> These latter effects could be causally related to prevention of myocardial damage in ischemia. Iloprost also has coronary vasodilating action,<sup>11)</sup> and it was recently reported to have increased regional myocardial blood flow (MBF) in nonischemic but not ischemic areas of treated animals.<sup>5,11)</sup> However, contrasting results were published by Farber et al., who reported no significant differences in MBF between iloprost-treated and control canine hearts in both ischemic and nonischemic regions.<sup>4)</sup>

In general, a number of energy metabolic changes such as mitochondrial dysfunction and loss of high energy phosphates are observed in ischemic myocardium.<sup>12)</sup> Pissarek et al. reported administration of iloprost to result in an appreciable reduction in the loss of high energy phosphates in ischemic dog myocardium.<sup>13)</sup> On the other hand, Ferrari et al. reported that iloprost treatment did not alter the myocardial depletion of ATP and creatine phosphate, although the ischemia-induced deterioration of mitochondrial function was attenuated.<sup>3)</sup> Little attention has been directed toward the influence of iloprost on the relation between myocardial energy metabolism and MBF. We previously described that using anesthetized dogs with coronary ligation ischemia-induced mitochondrial dysfunction and degradation of high energy phosphates

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depended on the degree of decrease in the MBF to the area involved. The purpose of the present study was to assess the effects of iloprost on metabolic and blood flow parameters to clarify the mechanism underlying its protection against myocardial dysfunction under ischemia.

## MATERIALS AND METHODS

### *Animal preparation*

Adult mongrel dogs of both sexes, weighing 7 to 13 kg, were used in the investigation. They were anesthetized by intraperitoneal administration of 50 mg/kg of pentobarbital, endotracheal intubation was performed, and their lungs were ventilated with room air. Aortic blood pressure was monitored through a polyethylene catheter passed retrograde from the femoral artery. The chest was opened with a fourth intercostal incision, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was dissected free, immediately distal to the first diagonal branch for ligation (Fig. 1).

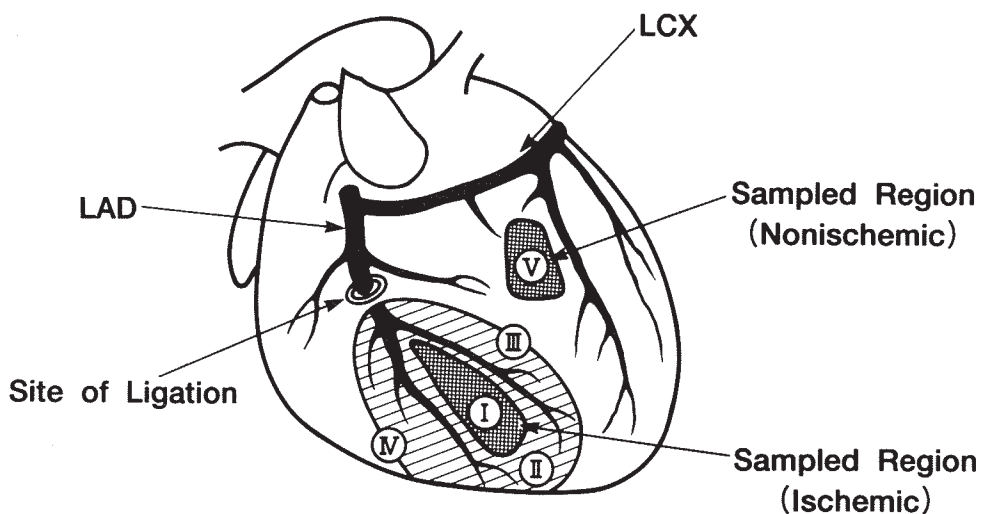


Fig. 1. Schematic diagram of the main coronary arteries in the canine heart. The site of ligation, immediately distal to the first diagonal branch, is indicated by an arrow, and a hoop hatching shows the ischemic area. Platinum electrodes, labeled I to V, were inserted into the left ventricular wall for measurement of regional myocardial blood flow. Tissue samples were subsequently taken from the sites of electrode insertion for the determination of ATP and creatine phosphate. Myocardial mitochondria were sampled from the dotted ischemic and nonischemic areas. LAD: left anterior descending coronary artery, LCX: left circumflex coronary artery.

### *Experimental protocol*

Dogs were subjected to 60 min of myocardial ischemia by LAD ligation using a silk suture. Iloprost (donated by Eisai Co., Ltd., Tokyo) was dissolved in physiological saline. Doses of 0.1 (n=8) or 0.2 (n=8)  $\mu\text{g}/\text{kg}/\text{min}$  were infused into the right femoral vein. Iloprost administration was started 30 min before the onset of ischemia and the infusion was continued throughout the 90 min observation period. Control animals (n=5) received vehicle at the same infusion rate. After the administration of 0.1 and 0.2  $\mu\text{g}/\text{kg}/\text{min}$ , one of eight and four of eight dogs,

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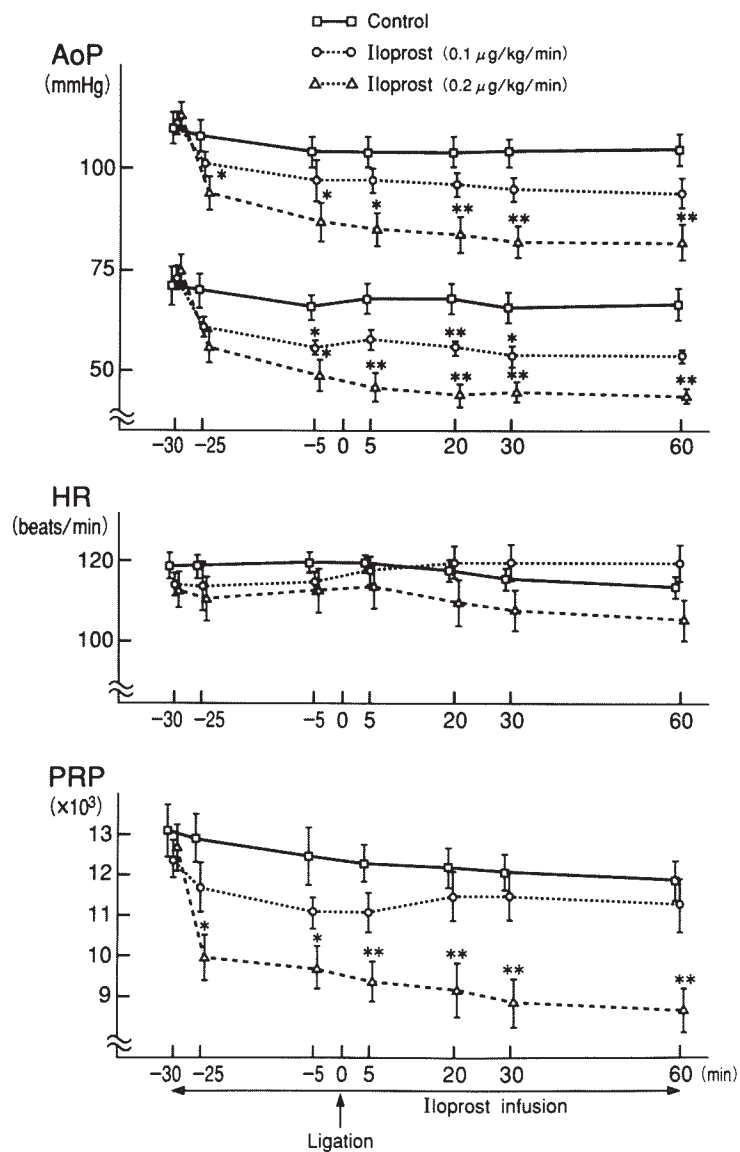


Fig. 2. Effects of intravenous administration of iloprost (control,  $n=5$ ;  $0.1 \mu\text{g}/\text{kg}/\text{min}$ ,  $n=7$ ;  $0.2 \mu\text{g}/\text{kg}/\text{min}$ ,  $n=4$ ) on aortic pressure (AoP), heart rate (HR) and pressure rate product (PRP). Graphs show mean  $\pm$ SE values.

\* $p < 0.05$ , \*\* $p < 0.01$  as compared with respective control values.

respectively, died and these were excluded from the data analysis. There were no significant differences in systolic aortic pressure between iloprost  $0.1 \mu\text{g}/\text{kg}/\text{min}$  and control groups, whereas in the iloprost  $0.2 \mu\text{g}/\text{kg}/\text{min}$  group significantly lower values were found (Fig. 2). Therefore, the  $0.1 \mu\text{g}/\text{kg}/\text{min}$  dose was chosen for further investigation.

In the second study, twenty dogs were divided into two groups: one group was given an infusion of iloprost at the dose of 0.1  $\mu\text{g}/\text{kg}/\text{min}$  (iloprost group); the other received the vehicle alone (control group). MBF values were measured before and 5, 20, 30 and 60 min after ligation. After 60 min of LAD occlusion, myocardial specimens were taken from various areas where MBF values were measured, for determination of tissue ATP and creatine phosphate (CP) and mitochondrial isolation.

#### *MBF measurements*

MBF was measured by polarographic recording of hydrogen desaturation with platinum electrodes as described by Aukland *et al.*<sup>16)</sup> Wire-type platinum electrodes, 0.08 mm in diameter, were introduced into various heart muscle sites (shown in Fig. 1) and fixed into the left ventricular wall in the middle of the ischemic area (I), the border at the visible ischemic edge (II, III, IV), and the nonischemic area (V). The electrodes were connected to a tissue rheometer (UH Meter PHG 201, Unique Medical Co., Ltd., Tokyo) and the hydrogen gas method was followed. Inhaled gas was changed from room air to a gas mixture containing about 8% hydrogen, and after 3 min, the gas was switched back to room air, and the hydrogen in the myocardium was washed out. The MBF was calculated by the formula based on Kety's approach to blood-tissue exchange of inert gases.<sup>17)</sup>

#### *High energy phosphate determinations*

Sixty min after LAD ligation, specimens of cardiac muscles corresponding to the respective sites of electrode implantation were collected in about one sec, using a biopsy drill with a diameter of 3 mm,<sup>18)</sup> and rapidly frozen in liquid nitrogen. The myocardial middle layer was used as a sample for determination of tissue ATP and CP. Each frozen myocardial tissue sample was weighed and homogenized with the addition of 2.5 ml of 7% perchloric acid per 100 mg tissue at 0°C. After deproteinization, tris buffer containing 4N-KOH was added to the homogenate to neutralize the perchloric acid, and the sample was centrifuged at 3000 rpm for 10 min. The ATP and CP contents were determined by a modification of McElroy-Strehler's firefly luminescence method using a firefly lantern extract (FLE 50, Sigma Chemical Co., St. Louis, Mo.).<sup>19,20)</sup> The measurements were made with a bioluminescence reader (Model BLR-101 C, Aloka Co., Tokyo).

#### *Mitochondrial function determinations*

Sixty min after LAD ligation, cardiac muscles of the ischemic area (I) and nonischemic area (V) conforming to the regions of MBF determination, were sampled, and the mitochondria were isolated using alkaline protease according to Hatefi's method.<sup>21)</sup> The mitochondrial sample (0.3 ml) was added to 2.8 ml of mannitol reaction solution (0.3 M mannitol, 10 mM potassium phosphate, 2.5 mM magnesium chloride, 10 mM potassium chloride, 0.25 mM EDTA), and 0.1 ml of succinic acid (0.2 M) as the substrate and 0.05 ml of ADP (0.01 M) was added. The respiratory activity of mitochondria was assessed using a bioxygraph (Sensonix Japan Co.). Its respiratory control index (RCI) was calculated.

#### *Statistical analysis*

The results presented are mean $\pm$ SE values. Statistical analysis was carried out using Student's *t* test. The probability was considered significant if less than 0.05.

## RESULTS

*Hemodynamics*

Systolic aortic pressure in the iloprost 0.2  $\mu\text{g}/\text{kg}/\text{min}$  group was found to be significantly depressed as compared with the control group values (Fig. 2). Systolic aortic pressure in the iloprost 0.1  $\mu\text{g}/\text{kg}/\text{min}$  group showed a tendency to decrease, but the reduction was not significant at any time point. Diastolic blood pressure was significantly lower with both 0.2 and 0.1  $\mu\text{g}/\text{kg}/\text{min}$  doses of iloprost. There were no significant differences in heart rate between the three groups throughout the experiment, and no significant changes due to LAD ligation were observed in aortic pressure and heart rate.

*Regional myocardial blood flow*

The degree of decrease in MBS in the ischemic (I), border (II, III, IV) and nonischemic (V) areas is not always uniform.<sup>14,15</sup> The MBF of the middle layer of the left ventricular wall in sham-operated animals was  $103 \pm 22$  ml/min/100g, and taking the mean-2SD of this value as the lower limit of normal, values of 60 and above were regarded as nonischemic areas. The severely ischemic area was the region of the myocardium with MBF values of less than 20, the moderately ischemic area had MBF values of 20 to 40, and the mildly ischemic area had MBF values of 40 to 60.

Fig. 3 shows the time-course changes in MBF divided into four subgroups according to the MBF level at 5 min after ligation. No significant differences between the iloprost and control animals were observed within subgroups.

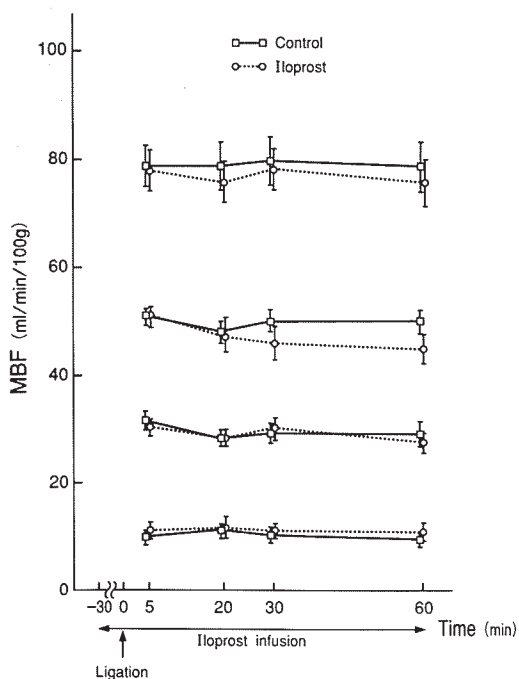


Fig. 3. Effects of intravenous administration of iloprost (0.1  $\mu\text{g}/\text{kg}/\text{min}$ ) on regional myocardial blood flow (MBF) in four subgroups divided according to the level of MBF at 5 min after coronary ligation (different subgroup for each increment of MBF of 20 ml/min/100g). Graphs show mean  $\pm$  SE values.

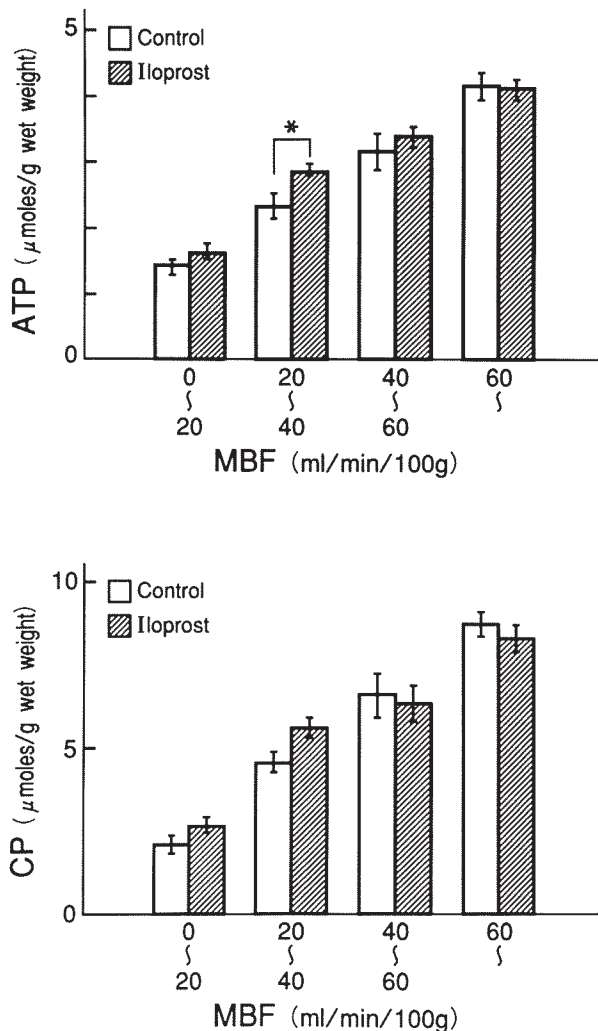


Fig. 4. Effects of intravenous administration of iloprost ( $0.1 \mu\text{g}/\text{kg}/\text{min}$ ) on myocardial contents of high energy phosphates. Histograms show mean  $\pm$  SE contents of ATP and creatine phosphate (CP) 60 min after coronary ligation in various areas of canine myocardium divided into four subgroups. \* $p < 0.05$

#### *Myocardial high energy phosphates*

Fig. 4 illustrates ATP and CP contents 60 min after ligation, in various myocardial areas divided into the four subgroups according to the level of MBF at 5 min after ligation. ATP concentration in the severely ischemic area showed a tendency to increase in the iloprost group. The moderately ischemic area demonstrated a significantly higher ATP value in the iloprost group ( $2.82 \pm 0.17$ ) as compared with that in the control group ( $2.32 \pm 0.23$ ). CP levels in both the severely and moderately ischemic areas exhibited a significant increase in the iloprost group as compared with those in the control group.

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Table 1. Respiratory Function of Mitochondria Isolated from Nonischemic and Ischemic Areas.

	Respiratory Control Index	
	Control	Iloprost
Nonischemic	4.45±0.20	4.38±0.19
Ischemic	1.59±0.08	2.18±0.13**

Data are mean±SE values for ten hearts.

\*\*p<0.01. Significantly increased as compared with the control group ischemic area.

#### *Mitochondrial respiratory function*

The RCIs in the ischemic areas of the iloprost group showed significantly higher values as compared with those of the control group. In contrast, there was no significant difference for nonischemic tissues (Table 1).

## DISCUSSION

In the present study, the prostacyclin analogue, iloprost, was demonstrated to ameliorate the deterioration of energy metabolism in ischemic myocardium without affecting MBF.

Lefer et al. made the original suggestion that prostacyclin could possibly exert beneficial effects on ischemic myocardium.<sup>22)</sup> However, utilization of prostacyclin is limited by its chemical instability and therefore attention has lately been focused on analogues that are characterized by a greater stability in aqueous solutions. Among these, iloprost has been proposed as particularly effective for protecting ischemic myocardium.<sup>2-5)</sup>

There are a variety of metabolic alterations in the ischemic myocardium such as loss of high energy phosphates, disturbance of mitochondrial function and structure, and abnormal accumulation of metabolites.<sup>12,14,15,23)</sup> Therefore, it seems that the following are conceivably involved in the mechanisms of maintenance of myocardial viability by iloprost: (1) increased blood flow to the ischemic myocardium, (2) decreased myocardial oxygen consumption resulting from a depression in the pressure-rate product, and (3) direct effects on cardiac energy metabolism.

Iloprost may act as a potent vasodilator<sup>1,24)</sup> and has been reported to produce dose-dependent decrease in blood pressure.<sup>1,25)</sup> Indeed, in the present study, systolic aortic pressure in dogs receiving iloprost at 0.2 µg/kg/min was significantly depressed. However, there was no significant difference in this parameter between the iloprost 0.1 µg/kg/min and the control groups and, as a result, the pressure-rate product was similar in the two groups. This is in line with earlier reports.<sup>5,11)</sup> No increase in MBF in ischemic areas was observed<sup>4,5,11)</sup> despite a coronary vasodilating action of iloprost.<sup>1)</sup> Similar results were found in the present study in both ischemic and nonischemic areas. Thus iloprost did not affect the MBF.

In the present investigation, determination of high energy phosphate contents in canine myocardium corresponding to the sites of MBF measurement demonstrated clear improvement in both ATP concentration in moderately ischemic areas and CP concentration in severely and moderately ischemic areas. Moreover, iloprost preserved mitochondrial respiratory function in ischemic myocardium. These beneficial effects may depend on decreased blood pressure. However, a number of studies have reported that changes in myocardial oxygen demand appeared to play only a minor role in the cardioprotective effects of iloprost.<sup>3,4,10)</sup> While there is some controversy concerning the influence of iloprost on energy metabolism in this situation,<sup>3,13)</sup> our results do suggest that this aspect is relevant to its cardioprotective effects in acute ischemia.

Since the potential involvement of iloprost has drawn much attention, mechanistic aspects have been extensively studied. Activation of platelets and formation of aggregates in obstructed coronary vessels might considerably decrease the coronary perfusion to the ischemic area, and iloprost, in a dose-dependent fashion, inhibits both the primary and secondary wave of ADP-induced platelet aggregation in man.<sup>6,8)</sup> On the other hand, another chemically stable carbacyclin analogue demonstrates considerable cardioprotective activity in acute myocardial ischemia of the cat that can be explained by factors other than antiplatelet effects or improvement of regional perfusion to the damaged myocardium.<sup>26)</sup> Smith et al. suggested that iloprost preserves biochemical and functional myocardial integrity in ischemic rabbit hearts by a membrane stabilizing mechanism.<sup>9)</sup> Furthermore, the agent exerts a definite cytoprotective effect due to its membrane sparing action by prevention of ischemia-induced loss of phospholipids.<sup>10)</sup> Another possibility involves reduced peroxide formation. Thiernemann et al. described that iloprost attenuated loss of superoxide dismutase-specific activity in cat hearts subjected to coronary occlusion and reperfusion, suggesting an effect on polymorphonuclear cells.<sup>27)</sup>

In conclusion, intravenous infusion of iloprost brought about preservation of myocardial energy metabolism without altering MBF in ischemic myocardium. Our findings indicate that iloprost therapy of acute myocardial ischemia may have potential clinical application.

## ACKNOWLEDGEMENTS

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