# Effects of Prostacyclin Analogue, OP-2507, on Function and Metabolism in the Ischemic Working Rat Heart

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We examined the effects of a new stable prostacyclin analogue, OP-2507, on myocardial function and metabolism in the ischemic working rat heart preparation. The hearts were perfused with Krebs-Henseleit bicarbonate (KHB) buffer, and whole heart ischemia was induced by one-way aortic valve for 15 min follows by reperfusion for 30 min. In the treated hearts, OP-2507, 20 ng·ml$^{-1}$, was administered to KHB buffer from the beginning to the end of experiment. During ischemia, coronary flow in the OP-2507 group increased significantly more than that in the control group. The mechanical performance of both groups was impaired after ischemia. However, the recovery of coronary flow, cardiac output, peak systolic pressure and LV $dP/dT_{max}$ was significantly higher in the treated group than in the control group. The incidence of ventricular fibrillation during reperfusion was 100% and 25% in the control and the OP-2507 groups, respectively. Myocardial ATP content was significantly higher in the treated hearts than that in the control hearts. These results indicate that this stable prostacyclin analogue is beneficial in myocardial ischemia, even without its well known action of preventing platelet aggregation. (Key words: myocardial ischemia, myocardial metabolism, prostacyclin analogue OP-2507, working rat heart preparation)

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Prostacyclin has several biological activities that include prevention of platelet aggregation[1,2], coronary vasodilation[3,4], stabilization of lysosomal membranes[5,6], and inhibition of thromboxane generation[5]. There has been much interest in prostaglandins and thromboxanes as mediators of unstable angina pectoris and acute myocardial infarction[7,8]. In animal experiments, prostacyclin was shown to reduce myocardial damage against ischemia and reperfusion[6,9,10]. But the instability of prostacyclin limits its clinical application during long ischemic period.

Recently, a stable prostacyclin analogue, OP-2507 [15-cis-(4-propylcyclohexyl)-16,17,18,19,20-pentanor-9-deoxy-9$\alpha$, 6-nitrilo-$PGF_1$ Methyl Es-

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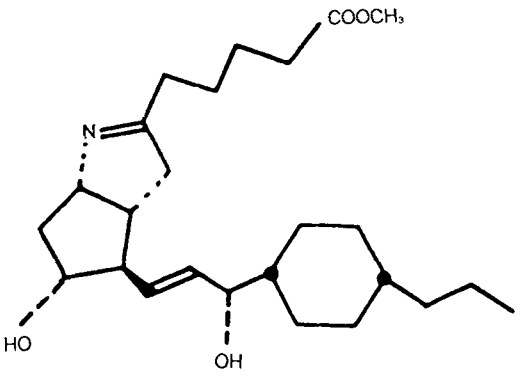


Fig. 1. Chemical structure of OP-2507.

ter] (chemical structure shown in figure 1), was demonstrated to have several prostacyclin-mimetic activities, both *in vitro* and *in vivo*[11–13]. The aim of this study was to determine whether this stable prostacyclin analogue, OP-2507, could reduce tissue injury occurring during myocardial ischemia in isolated rat hearts.

## Methods

These experiments were approved by the Animal Ethical Committee of the Yamanashi Medical College. Sixteen 3-month-old male Wistar rats weighting 280–320g were used. The animals were anesthetized with sodium pentobarbital 50 $mg \cdot kg^{-1}$ b.w. intraperitoneally. The heart was then rapidly excised and put into ice-cold saline, which stopped the heart activity within seconds. The aorta was cannulated distal to the aortic valve and the heart was immediately perfused retrogradely through the aorta. Non-recirculating modified Krebs-Henseleit bicarbonate buffer was used as preperfusate. The perfusate was maintained at 37.0 ± 0.3°C and contained (mM): NaCl 118, KCl 4.7, $CaCl_2$ 3.0, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25, di-NaEDTA 0.5, and glucose 11. During the retrograde perfusion, the left atrium was connected via a pulmonary vein to an angled steel cannula. The remaining pulmonary vein were ligated to avoid leakage. After this preliminary perfusion, the heart was converted to working preparation by perfusing the left atrium and releasing the aortic outflow for a stabilization period of approximately 10 min. A non-recirculation Krebs-Henseleit bicarbonate buffer was used also during working heart systems. In the treated group, the perfusate contained OP-2507, 20 $ng \cdot ml^{-1}$, and was used to the end of the experiment.

Left ventricular pressure (LVP) was measured with a transducer (P10EZ, Gould, U.S.A.) connected to a thin catheter (18G, Intramedicut Catheter, Argyle, U.S.A.) inserted into the left ventricle through the mitral valve from angled steel cannula in the left atrium. Rates of tension development (LV $dP/dt_{max}$) were measured from the derivatives of LVP obtained electronically.

Aortic pressure was recorded via a Gould P10EZ transducer on a polygraph (Nihonkohden, Japan). Aortic outflow was recorded with an electromagnetic blood flow meter (MFV-3200, Nihonkohden, Japan). Coronary flow was measured by timed collection of the pulmonary artery outflow and surface runoff of the heart resulting from coronary sinus and Thebesian vessel drainage. Cardiac output was considered as the sum of the aortic and coronary outflows. At no time was the coronary effluent recirculated.

The solution was equilibrated with a gas mixture of 95% $O_2$ and 5% $CO_2$. Aortic oxygen tension was estimated by sampling perfusate from the atrial bubble trap on the left atrial line with a gas-tight syringe. For measurement of oxygen tension of coronary effluent, a catheter was placed in the pulmonary artery, from which samples were obtained with a gas-tight syringe. The oxygen tension was measured in an intermittently self-calibrating blood

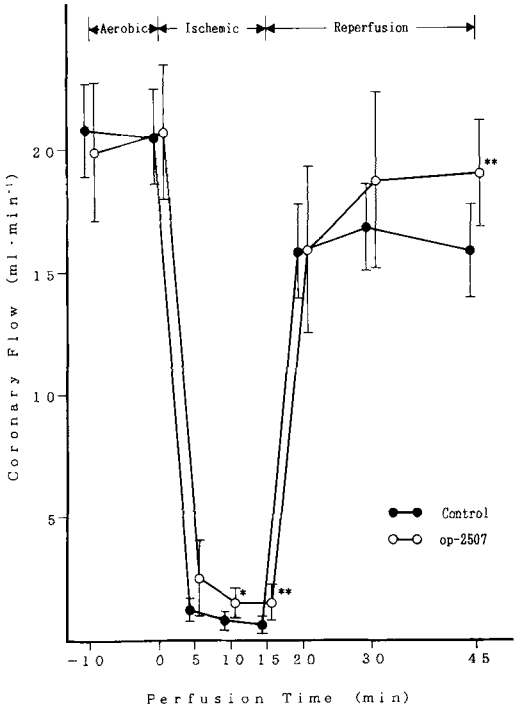


**Fig. 2.** Changes in coronary flow of control (closed circles) and OP-2507 treated hearts (open circles) as a function of time. The X-axis represents perfusion time in min. At zero time in the figure, ischemia was induced by one-way aortic valve procedure. Perfusion as an ischemic heart was continued for 15 min followed by reperfusion for 30 min. Each point represents mean ± SD for 8 hearts. $*P < 0.02$, $**P < 0.01$ as compared with control.

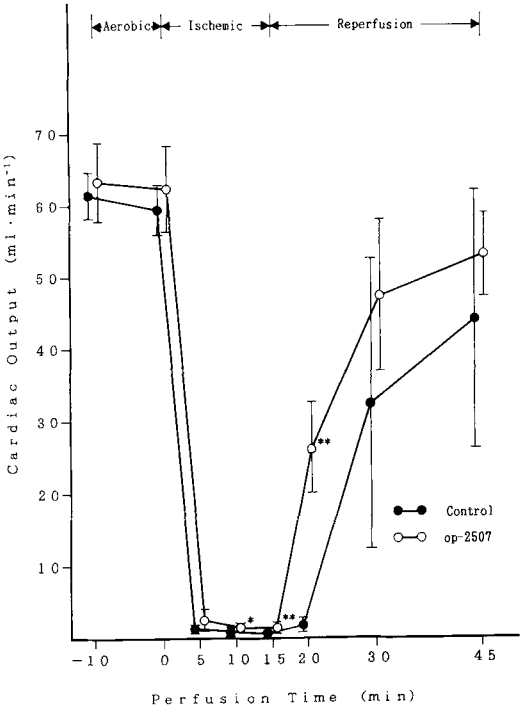


**Fig. 3.** Changes in cardiac output of control (closed circles) and OP-2507 treated hearts (open circles) as a function of time. The cardiac output was considered as the sum of the aortic and coronary outflows. At zero time, whole heart ischemia was induced for 15 min followed by reperfusion for 30 min. Each point represents mean ± SD for 8 hearts. $*P < 0.02$, $**P < 0.01$ as compared with control.

**gas analyzer system (Instrumentation Laboratory Model 1306, U.S.A.). Myocardial oxygen consumption, $M\dot{V}O_2$ (mmoles·min$^{-1}$·gram$^{-1}$), was calculated as follows:**

$$M\dot{V}O_2 = (P_{O_{2LA}} - P_{O_{2PA}}) \times \text{coronary flow/gram heart dry weight} \times b/22.4,$$

**Where: $P_{O_{2LA}} = P_{O_2}$ in the perfusate entering left atrium; $P_{O_{2PA}} = P_{O_2}$ in the venous coronary effluent; b = Bunsen coefficient, 0.0239 ml $O_2$/ml $H_2O$ × 760 mmHg, which is the solubility of oxygen at 37°C; and 22.4 = conversion factor from ml $O_2$ to mmoles $O_2$.**

**Ten min after stabilization period, whole heart ischemia was induced by clamping one-way aortic valve bypass for 15 min. The aortic cannula was provided with sidearms both above and below the one-way valve. These sidearms were connected by a short length of Tygon tubing, which provided a bypass around the one-way valve for control perfusions. The lower sidearm was also connected to a pressure transducer and to the preperfusion reservoir. Ischemia was induced in this preparation by simply clamping the bypass tube. Since the largest fraction of coronary flow occurs during**

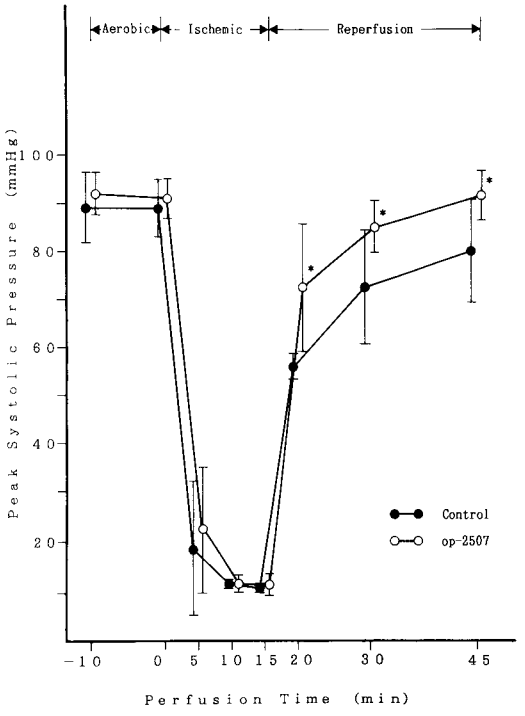


**Fig. 4.** Changes in peak systolic pressure of control (closed circles) and OP-2507 treated hearts (open circles) as a function of time. At zero time, whole heart ischemia was induced for 15 min followed by reperfusion for 30 min. Each point represents mean ± SD for 8 hearts. $*P < 0.02$ as compared with control.

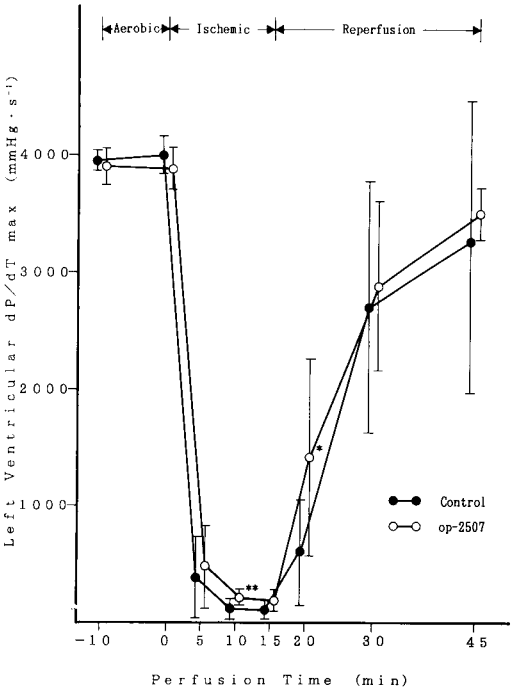


**Fig. 5.** Changes in left ventricular dP/dT max of control (closed circles) and OP-2507 treated hearts (open circles) as a function of time. At zero time, whole heart ischemia was induced for 15 min followed by reperfusion for 30 min. Each point represents mean ± SD for 8 hearts. $*P < 0.05$, $**P < 0.02$ as compared with control.

diastole, this one-way valve severely restricted coronary perfusion, but did not influence aortic output or ventricular afterload[14]. Reperfusion of the hearts after this ischemic period of 15 min was performed by declamping the one-way aortic valve bypass tube and lasted for 30 min.

At the end of perfusion, hearts were quickly frozen by clamping with a Wollenberger clamp cooled in liquid nitrogen and freeze-dried for 6 days. An aliquot was extracted with perchloric acid and centrifuged at 3,000g. Concentrations of ATP and lactate were measured spectro-photometrically by standard techniques[15]. Another piece of freeze-dried sample was placed in 30% potassium hydroxide and digested at 100°C. Tissue glycogen was extracted, hydrolyzed and assayed as glucose equivalents[16]. The values were expressed as μmol per gram dry heart weight.

The data are expressed as means ± SD. Statistical significances between the groups were evaluated using non-paired Student's t-test. The incidence of ventricular fibrillation (Vf) was analyzed by a chi-square test. A probability of $P < 0.05$ was regarded as statistically significant.

## Results

Before ischemia, there were no significant differences in hemodynamic

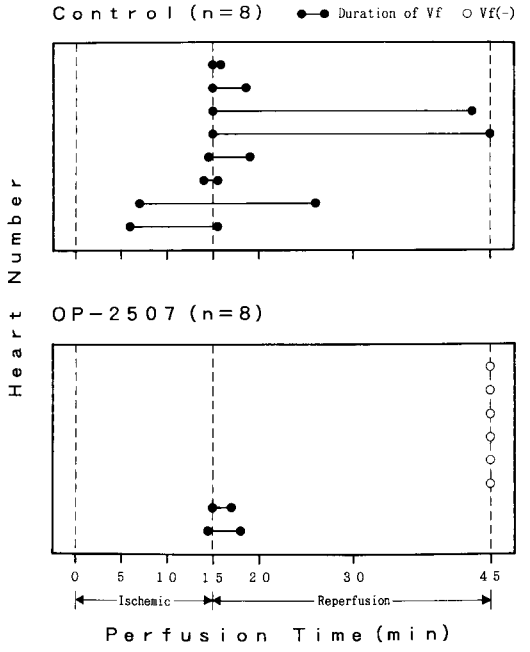


**Fig. 6.** Time of onset and duration of ischemia and reperfusion-induced ventricular fibrillation (Vf). The time of onset (closed circles in left end of each line) and the duration of Vf (distance between the left and right closed circles) are illustrated for individual heart. Open circles represent the individual heart where Vf did not occur during either ischemic or reperfused periods.

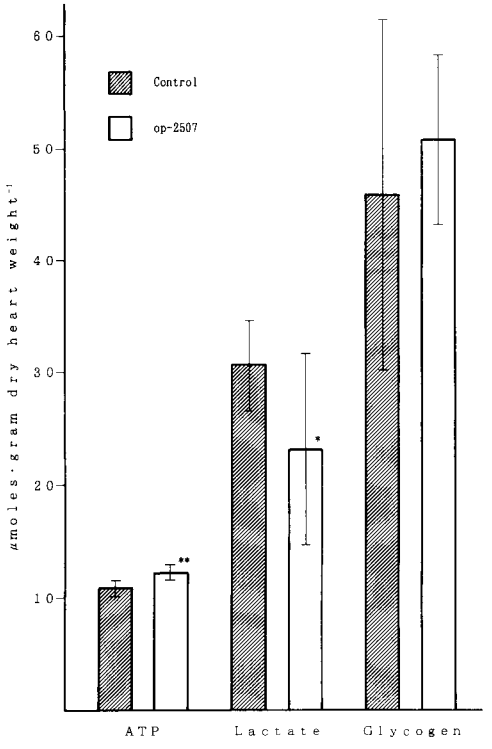


**Fig. 7.** Myocardial concentrations of ATP, lactate, and glycogen (n=8 each group). $*P < 0.05$, $**P < 0.01$ as compared witih control.

data between the treated and control groups. During ischemia, coronary flow in the treated group increased significantly more than that in the control group (fig. 2).

During reperfusion, there was no significant difference in heart rate between two groups (table 1). However, coronary flow, cardiac output, peak systolic pressure, rate pressure products and LV $dP/dT_{max}$ of the treated hearts recovered more rapidly than those of the control hearts (table 1, fig. 2–5). Upon reperfusion, Vfs were observed in all the control hearts. However, only two of eight treated hearts showed Vfs (fig. 6).

Although there were no significant differences in $M\dot{V}O_2$ and myocardial glycogen concentrations between the two groups, myocardial ATP content in the OP-2507 group was significantly greater than that in the control group, and lactate content in the OP-2507 group was significantly less than that in the control group (table 1, fig. 7).

## Discussion

In the present study, significant improvement of cardiac function by OP-2507, a stable prostacyclin analogue with a similar pharmacological profile to prostacyclin, was observed upon reperfusion following 15 min of ischemia. OP-2507 also increased myocardial ATP content and reduced myocardial lactate concentration. These findings imply that prostacyclin analogue, OP-2507, could improve func-

**Table 1.** Heart rate (HR), myocardial oxygen consumption ($M\dot{V}O_2$), and rate pressure products (RPP) in the working rat hearts

| Variables | Time ⊢ischemia⊣ −10 | 0 | 20 | 30 | 45 min |
|---|---|---|---|---|---|
| HR (beats·min$^{-1}$) | | | | | |
| Control | 318 ± 14(8) | 316 ± 15(8) | 131 ± 18(5) | 303 ± 22(6) | 303 ± 27(7) |
| OP-2507 | 311 ± 24(8) | 300 ± 16(8) | 182 ± 58(8) | 284 ± 26(8) | 277 ± 22(8) |
| RPP ($\times 10^2$ mmHg·min$^{-1}$) | | | | | |
| Control | 283 ± 23(8) | 282 ± 21(8) | 74 ± 9(5) | 237 ± 8(6) | 252 ± 18(7) |
| OP-2507 | 286 ± 20(8) | 273 ± 15(8) | 136 ± 63*(8) | 241 ± 22(8) | 253 ± 18(8) |
| $M\dot{V}O_2$ ($\mu$moles·min$^{-1}$·gram$^{-1}$) | | | | | |
| Control | 39 ± 10(8) | 38 ± 8(8) | 22 ± 5(8) | 30 ± 8(8) | 33 ± 9(8) |
| OP-2507 | 38 ± 5(8) | 40 ± 6(8) | 25 ± 5(8) | 34 ± 6(8) | 36 ± 5(8) |

Number of observations is shown in parentheses. Each value is the mean ± SD,
*$P < 0.05$ as compared with control.

tional recovery and that it metabolically enhance the glycolytic high energy phosphate generation after myocardial ischemia.

Although prostacyclin can protect the heart against ischemia *in vivo* by preventing platelets from aggregation[2,17–20], the perfusate of our preparation did not include platelets. This indicates that the participation of factors other than platelets may be attributed to protection of the myocardium by OP-2507. There are several possible explanations for the protective effects observed in our study.

First, in the present study, OP-2507 showed no significant effect on coronary flow at the dose employed during pre-ischemic control period. However, during ischemia and reperfusion, coronary flow was significantly higher than in the untreated ischemic hearts. Ribeiro et al.[10] have reported that the ratio of ischemic to normal myocardial blood flow was increased by prostacyclin. Our results also indicate that the prostacyclin analogue, OP-2507, produced coronary vasodilation which might allow favorable redistribution of coronary blood flow. Thus, it seems likely that enhancement of tissue perfusion is one of the factors in the recovery observed in the treated hearts.

Second, some investigators[5,6,21–24] have regarded this protective effect against ischemia as a direct cytoprotective effect, a membrane-stabilizing activity, and/or prevention of cell integrity. The precise molecular mechanism of the cytoprotective effect of prostacyclin is not completely understood at present. Cardiac lysosomes include several acid hydrolases including proteases and phospholipases. If these enzymes are released into the cytoplasm, they may contribute to the degradation of structural proteins and membrane phospholipids[6]. During ischemia, leakage of lysosomal enzymes is reported to occur before the irreversible damage of myocardium[25,26]. It has been also reported that prostacyclin is a potent stabilizer of lysosomes[5,6,19,27]. Therefore, it is possible that the potent stabilizing action of prostacyclin analogue, OP-2507, on lysosomes is another important aspect of the mechanism responsible for the cytoprotective effect during ischemia in the present study.

Third, the administration of OP-2507 had also beneficial actions on the arrhythmias induced by both coronary artery occlusion and reperfusion. The beneficial effect of OP-2507 on reperfusion-induced Vf is of considerable interest. In other studies, prostacyclin has been reported to reduce the incidence of Vf during coronary occlusion in dogs[10,28–31] and rats[29]. Coker et al.[32] have demonstrated that dazoxiben, a thromboxane synthetase inhibitor, has similar effects in reducing Vf following reperfusion of the ischemic myocardium. This suggests that the balance between thromboxane and prostacyclin in this situation may be related to the occurrence of arrhythmias. In addition, the antiarrhythmic effect of OP-2507 may be resulted from its antiadrenergic actions. It has been reported that prostacyclin prevents ischemia-induced increases in myocardial lactate and cyclic AMP[33] which increases immediately prior to Vf[34]. There are other reports that some of the actions of prostacyclin and analogues are linked to catecholamines[28,35–37].

In conclusion, a stable prostacyclin analogue, OP-2507, seems to have coronary vasodilator, cytoprotective, and antiarrhythmic actions and to counteract the deleterious effects, mechanical, metabolic and electrophysiologic effects, following reperfusion in the ischemic heart independent of its inhibitory effect on platelet aggregation. These results may suggest that OP-2507 could prove beneficial in the early stages of acute myocardial infarction.

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