# Protective Effects of Prostaglandin $I_2$ Analogues on CPK Release in Rat's Heart-lung Preparation

Satoshi KASHIMOTO, Toshihiro NAKAMURA, Takeshi OGUCHI*, Masaki KUME* and Teruo KUMAZAWA*

The effects of prostaglandin $I_2$ analogues ($PGI_2$-a: op-41483 and op-2507) on oxygen toxicity during hyperoxic perfusion were evaluated in an experiment on isolated rat heart lung preparation, with the release of creatine phosphokinase (CPK) in the perfusate blood. There were no significant differences in heart rate and right atrial pressure between $PGI_2$-a treated and untreated hearts. The CPK release from the heart with oxygen was significantly higher than that of the air ($P < 0.001$). However, the CPK release from the $PGI_2$-a treated hearts was significantly less than that from the untreated hearts ($P < 0.05$). These results indicate that $PGI_2$-a may prevent cell damage which was induced by hyperoxia. (Key words: creatine phosphokinase, heart lung preparation, hyperoxia, prostaglandin $I_2$ analogues)

(Kashimoto S, Nakamura T, Oguchi T, et al.: Protective effects of prostaglandin $I_2$ analogues on CPK release in rat's heart-lung preparation. J Anesth 5: 359 362, 1991)

Prostacyclin ($PGI_2$) and its analoque have been shown to be beneficial in traumatic shock[1], cerebral ischemia[2–4] and myocardial ischemia[5–8]. Their major effects are prevention of platelet aggregation[9–12] and coronary vasodilation[13,14]. However, Araki and Lefer[15] have indicated that $PGI_2$ might also protect the isolated heart perfused with platelet-free buffer solution at doses below those that effect vessel tone. This protective effect of $PGI_2$ *in vitro* might be related to a stabilization of cell membranes.

We have previously reported that superoxide dismutase (SOD) revealed protective effects on hyperoxic perfused heart by reducing the creatine phosphokinase (CPK) release[16]. It is, thus, important to examine the direct effects of the new stable prostaglandin $I_2$ analogues ($PGI_2$-a) on hyperoxic myocardial injury in an isolated rat's heart lung preparation where actions of $PGI_2$-a on the coronary vasculature and on platelet aggregation were eliminated.

## Materials and Methods

The experiment was performed in accordance with Guidelines for Animal Experiments, Yamanashi Medical College. The techniques used were identical to those used in our previous study[16]. Briefly, 72 male Wistar-Kyoto rats (300–330g) were anesthetized with 50 $mg \cdot kg^{-1}$ of pentobarbital intraperitoneally. A tracheostomy was performed, and constant volume (1.5 ml) intermittent positive pressure ventilation was instituted at a rate of 80 $breaths \cdot min^{-1}$ with

*Department of Anesthesia, Kohfu City Hospital, Yamanashi, Japan*

**Department of Anesthesiology, Yamanashi Medical College, Yamanashi, Japan*

*Address reprint requests to Dr. Satoshi Kashimoto: Department of Anesthesia, Kohfu City Hospital, 14-6, Saiwai-cho, Kohfu-city, Yamanashi, 400 Japan*

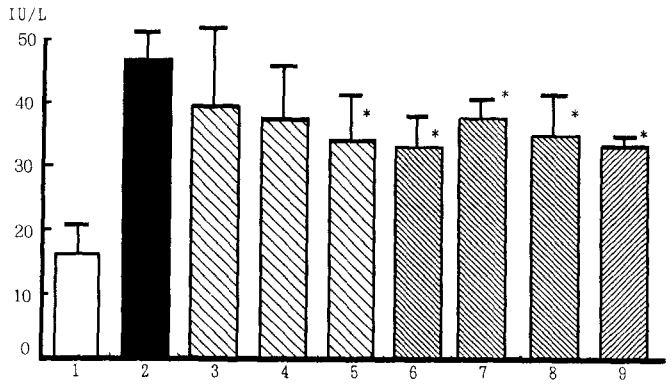


**Fig. 1.** The CPK release from the hearts.
1. none (air group);
2. saline (control);
3. op-41483, 3 ng·ml$^{-1}$;
4. op-41483, 30 ng·ml$^{-1}$;
5. op-41483, 300 ng·ml$^{-1}$;
6. op-2507, 3 ng·ml$^{-1}$;
7. op-2507, 30 ng·ml$^{-1}$;
8. op-2507, 300 ng·ml$^{-1}$;
9. op-2507, 3 ng·ml$^{-1}$·min$^{-1}$ to the end of the experiment.
(Each group: n = 8) *$P < 0.05$ vs control.

100% oxygen. The chest was opened and flooded with ice-cold saline and the heart was arrested during the preparation. Cannulae were inserted into the aorta and the superior and inferior venae cavae. The cannula of the superior vena cava was used to monitor right atrial pressure. A heart lung preparation was perfused with the perfusate (25 ml), containing red blood cells collected from another rat, and Krebs Ringer bicarbonate buffer (with hematocrit; 25%, pH; 7.4). The concentrations (mM) of the buffer constituents were as follows: NaCl 127, KCl 5.1, $CaCl_2$ 2.2, $KH_2PO_4$ 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15, and heparin. The perfusate pumped from the aorta, passing through a pneumatic resistance, was collected in a reservoir, warmed at 37°C throughout the experiment using a water jacket, and then returned to the inferior vena cava. No other organs except heart and lung were perfused.

The heart rate was recorded with a Nihonkohden's bioelectric amplifier AB-621G and the total blood flow rate was measured with an electromagnetic blood flow meter MFV-1200 (Nihonkohden Co. Ltd., Japan). Arterial pressure and right atrial pressure were measured with carrier amplifiers AP-621G using transducer TP-101T and LPU-0.1A (Nihonkohden Co. Ltd.), respectively.

All hearts were perfused at total blood flow rate of 30 ml·min$^{-1}$ and a systolic arterial pressure of 80 mmHg. Five min. after the start of the perfusion, saline or $PGI_2$-a (op-41483 or op-2507) was administered in the reservoir in the following nine groups (each group: n = 8): 1. none (air group); 2. saline (control); 3. op-41483, 3 ng·ml$^{-1}$; 4. op-41483, 30 ng·ml$^{-1}$; 5. op-41483, 300 ng·ml$^{-1}$; 6. op-2507, 3 ng·ml$^{-1}$; 7. op-2507, 30 ng·ml$^{-1}$; 8. op-2507, 300 ng·ml$^{-1}$; 9. op-2507, 3 ng·ml$^{-1}$·min$^{-1}$ to the end of the experiment.

Thirty min. after the start of perfusion, blood gas analyses were made and the perfusate was collected and analyzed for creatine phosphokinase (CPK) activity by the tetrazolium method.

Statistical analysis was made by one way analysis of variance followed by the Dunnett test for comparing with the control values. A probability of $P < 0.05$ was regarded as statistically significant. The data are given as means ± SD.

## Results

The mean values of $P_{O_2}$ of the perfusate in all groups except the group 1 (air: 98 ± 12 mmHg) ranged from 396 to 438 mmHg and revealed no significant differences. There were no significant differences in heart rate (244 ~ 314 beats·min$^{-1}$) and right atrial pressure (16 ~ 22 mm $H_2O$) among all groups. The CPK release from the heart with oxygen (group 2) was significantly higher than that of the air (group 1) ($P < 0.001$). However, the CPK releases from the hearts with op-48413, 300 ng·ml$^{-1}$ and all doses of op-2507 were significantly less than those of hearts in the control group (fig. 1).

## Discussion

Findings of the present study confirmed the evidence that hyperoxia induces cell damage and results in CPK release. This may be mediated in part by cytotoxic oxygen metabolites since SOD decreases the CPK release in the same preparation[16].

$PGI_2$, a metabolite of arachidonic acid, is produced primarily by the endothelial cells of blood vessels and is biochemically unstable. Thus, we used $PGI_2$-a (op-41483 and op-2507)[3,4,11,12]. Both op-41483 and op-2507 are stable $PGI_2$-a which have similar profiles of action as the natural $PGI_2$. In this study, op-41483, 300 $ng \cdot ml^{-1}$ and all doses of op-2507 decreased the CPK release from the hyperoxic perfused hearts. The finding indicates that the participation of factors other than platelets and coronary vasodilation may be attributed to protection of the myocardium by $PGI_2$-a. Some investigators[1,5,6,8,15,17] have regarded this action as a membrane-stabilizing activity, a direct cytoprotective effect, or prevention of cell integrity. The membrane-stabilizing effect of op-2507 is more potent than that of op-41483 (unpublished data). This may be why even the minimum dose of op-2507 showed the protective effect on the hyperoxic perfused heart.

There may be another reason that $PGI_2$-a has a free radical scavenging effect. However, whether this protection is the result of a free radical scavenging effect cannot be concluded from this study.

The tolerance of oxygen toxicity in various species is different[18]. Therefore, the results obtained from the rats cannot be extrapolated directly to humans. However, we conclude that the administration of 100% oxygen, even in a short time, may exert a deleterious effect on the heart, and that $PGI_2$-a may have a protective effect against it.

Acknowledgement: We wish to thank M. Koshimizu and T. Amemiya for their valuable technical assistance and Ono Pharma. Co. Ltd. for the gift of op-41483 and op-2507.

(Received Jan. 22, 1991, accepted for publication Mar. 12, 1991)

## References

1. Lefer AM, Sollott SL, Galvin MJ: Beneficial actions of prostacyclin in traumatic shock. Prostaglandins 17:761–767, 1979
2. Hallenbeck JM, Furlow TW: Prostaglandin $I_2$ and indomethacin prevent impairment of post-ischemic brain reperfusion in the dog. Stroke 10:629–637, 1979
3. Masuda Y, Ochi Y, Karasawa T, Hatano N, Kadokawa T, Okegawa T: Protective effect of a new prostacyclin analogue op-2507 against cerebral anoxia and edema in experimental animals. Eur J Pharmacol 123:335–344, 1986
4. Terawaki T, Takakuwa T, Iguchi S, Wakitani K, Kira H, Okegawa T, Kawasaki A, Masuda Y: Effect of a prostacyclin analog op-2507 on acute ischemic cerebral edema in cats. Eur J Pharmacol 152:63–70, 1988
5. Ogletree ML, Smith JB, Nicolaou KC, Lefer AM: Beneficial actions of prostacyclin ($PGI_2$) in myocardial ischemia. Fed Proc 37:566, 1978
6. Lefer AM, Ogletree ML, Smith JB, Silver MJ, Nicolaou KC, Barnette WE, Gasic GP: Prostacyclin: A potentially valuable agent for preserving myocardial tissue in acute myocardial ischemia. Science 200:52–54, 1978
7. Ribeiro LG, Brandon TA, Hopkins DG, Reduto LA, Taylor AA, Miller RR: Prostacyclin in experimental myocardial ischemia: Effects on hemodynamics, regional myocardial blood flow, infarct size and mortality. Am J Cardiol 47:835–840, 1981
8. Schrör K, Darius H, Addicks K, Koster R, Smith EF III: $PGI_2$ prevents ischemia induced alterations in cardiac catecholamines without influencing nerve-stimulation-induced cathecholamine release in nonischemic conditions. J Cardiovasc Pharmacol 4:741–748, 1982
9. Moncada S, Gryglewski R, Bunting S, Vane JR: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263:663–665, 1976
10. Aiken JW, Gorman RR, Shebuski RJ: Prevention of blockage of partially obstructed coronary arteries with prostacyclin correlates with inhibition of platelet aggregation. Prostaglandins 17:483–494, 1979

11. Fujitani B, Wakitani K: Studies on antiplatelet effect of op-41483, a prostaglandin $I_2$ analog, in experimental animals: I. Effect on platelet function and thrombosis. Jap J Pharmacol 52:123–130, 1990
12. Fujitani B, Wakitani K: Studies on antiplatelet effect of op-41483, a prostaglandin $I_2$ analog, in experimental animals: II. Mechanism of the its antiplatelet effect. Jap J Pharmacol 53:25–33, 1990
13. Dusting GJ, Moncada S, Vane JR: Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13:3–15, 1977
14. Ogletree ML, Smith JB, Lefer AM: Actions of prostaglandins on isolated perfused cat coronary arteries. Am J Physiol 235:H400–H406, 1978
15. Araki H, Lefer AM: Role of prostacyclin in the preservation of ischemic myocardial tissue in the perfused cat heart. Circ Res 47:757–763, 1980
16. Kashimoto S, Kumazawa T: Protective effects of superoxide dismutase against oxygen toxicity in rat's heart lung preparation. Jap Circ J 51:1022-1026, 1987
17. Schrör K, Ohlendorf R, Darius H: Beneficial effects of a new carbacyclin derivative, ZK 36 374, in acute myocardial ischemia. J Pharmacol Exp Ther 219:243–249, 1981
18. Frank L, Bucher JR, Roberts RJ: Oxygen toxicity in neonatal and adult animals of various species. J Appl Physiol: Respirat Environ Exercise Physiol 45:699–704, 1978