

# Effects of Ischemic Preconditioning on Myocardial Protective on Cardiac Surgery: Possibility of Ischemic Preconditioning and Adenosine Administration

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**Subject:** We evaluated the efficacy for concomitant use of ischemic preconditioning (IPC) and cardioplegic arrest with adenosine premedication on myocardial protection.

**Methods:** Twenty-one pigs were divided into three groups: 1) control group, 2) IPC group which had IPC, 3) IPC+adenosine triphosphate (ATP) group which had an administration of 140  $\gamma$  of ATP (Adetphos, Kowa, Tokyo, Japan) during IPC. IPC was employed by 3 minutes of aortic cross clamping and 5 minutes of reperfusion. After cardioplegic arrest, the hemodynamical state was observed during 60 minutes of reperfusion. Serum adenosine, troponin-T, E-max, and Tau (the time constant of early diastolic left ventricular pressure decay) were compared.

**Results:** Serum adenosine levels and at the end of IPC and 60 minutes reperfusion were significantly higher in the IPC and IPC+ATP groups than the control group. Comparison of the myocardial contractile force indicator E-max showed that the IPC and IPC+ATP groups showed significantly higher recovery rates of myocardial contractile force than the control group. Tau was the lowest in the IPC+ATP group than the other groups. In the histopathological study, the control group showed widely distributed hypercontraction bands and waving degeneration of myofibrils. On the other hand, the structure of myofibrils was well preserved in the IPC and IPC+ATP groups.

**Conclusions:** The concomitant use of IPC enhanced the effect of a myocardial protective solution. However, the administration of adenosine during IPC did not show any further advantage than IPC along. (*Ann Thorac Cardiovasc Surg* 2003; 9: 307–13)

**Key words:** ischemic preconditioning, cardioplegic arrest, adenosine administration

## Introduction

In recent years, the use of cardioplegia has stabilized the postoperative results of myocardial protection during heart surgery. However, patients who have undergone prolonged cardioplegic arrest with cardioplegia are susceptible to low cardiac output syndrome and various arrhythmias, which increase the incidence of postoperative complications. On the other hand, the myocardial protective effect of ischemic preconditioning (IPC), a phenomenon in which, once subjected to ischemia, the myocardium ac-

quires resistance to subsequent ischemia, has attracted attention. Few studies have investigated the combined use of cardioplegic arrest and IPC, and there is no consensus on the subject.

Studies have reported that adenosine is involved in the mechanism of the myocardial protective effect of IPC,<sup>1)</sup> and gradually elucidated it. In this study, we investigated the effects of IPC alone or in combination with adenosine premedication on conventional cardioplegic arrest in terms of cardiac function, blood chemistry, hematology, biochemistry, and histopathology.

## Materials and Methods

Twenty-one pigs weighing 45.2 $\pm$ 3.8 kg (43.5–48.2 kg) were anesthetized by intramuscular injection of 20 mg/kg of pentobarbital and 10 mg/kg of ketamine hydrochloride.

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ride as premedication and a maintenance dose of 1 mg/kg/h of ketamine hydrochloride. After tracheostomy, respiration was controlled at 14/min, a tidal volume of 12 ml/kg, and an oxygen concentration of 40% with a ventilator (Servo Ventilator 900D, Siemens-Elcoma AB, Stockholm, Sweden). A pigtail catheter (TERUMO Corporation, Tokyo, Japan) was inserted from the right femoral artery to measure arterial pressure. A Sones catheter (TERUMO Corporation) was inserted from the right internal jugular vein, and placed in the coronary sinus under X-ray fluoroscopy to obtain blood. An intravenous route for fluid therapy through the left jugular vein was established. A conductance catheter (2012-6-27-p, Alpha Medical Instruments Inc., CA, USA) and a catheter with a pressure sensor (Centron Inc., Roden, the Netherlands) were inserted from the left and right carotid arteries, respectively, and placed in the left ventricle to construct left ventricular pressure-volume curves. The chest was opened in the supine position by median sternotomy and, after systemic heparinization with 300 U/kg, a right atrial venous cannula (TF-024-L, Research Medical Inc., NY, USA) and an ascending aortic arterial cannula (A211-5.2, Stockert, Munich, Germany) were inserted and secured, then a gyro pump (Kyocera Corporation, Kyoto, Japan) and an oxygenator (HPO-20H-C, Senkoikogyo Corporation, Tokyo, Japan) were placed for cardiopulmonary bypass (CPB). An aortic cannula (Medtronic Inc., NW, USA) was inserted into the base of the aorta, and used as an infusion route and vent for the myocardial protective solution and adenosine. CPB was performed at a flow rate of 80 ml/min/kg. Blood was pumped to the ascending aorta at 37°C, and IPC was performed during CPB by twice repeating 3 minutes of ascending aortic clamping and 5 minutes of reperfusion after declamping. Subsequently, after induction of ventricular fibrillation, the aorta was clamped; a myocardial protective solution (Myotector, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was infused; the aorta was declamped again after 40 minutes of cardioplegic arrest; then 60 minutes of reperfusion was performed (Fig. 1). Four degree of St. Thomas' solution was initially administered (30 ml/kg). An additional dosage of cardioplegic solution was unnecessary for all the cases. Blood samples were collected from the coronary sinus at 4 timepoints: 1) after initiation of CPB (pre), 2) immediately before ascending aortic clamping (IPC-end), 3) at the start of reperfusion (reperfusion), and 4) 60 minutes after reperfusion (R-60 min). Adenosine and troponin-T were measured as indicators of myocardial protective effect and myocardial

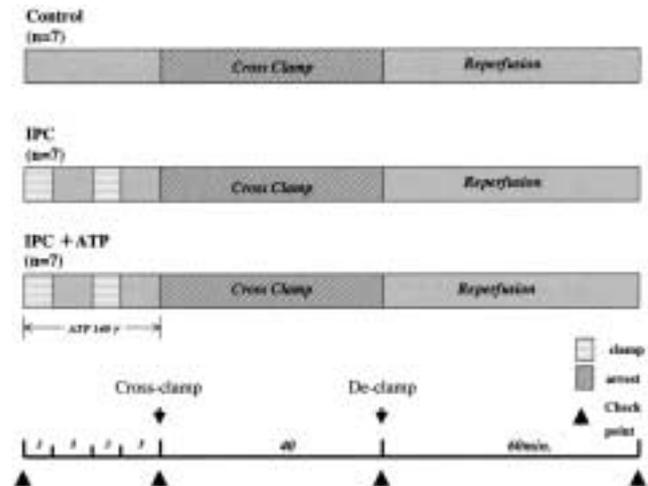
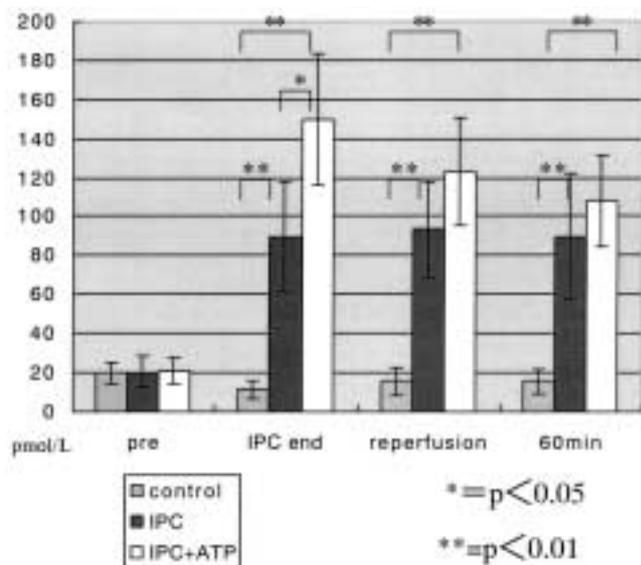


Fig. 1. Protocol of experiments.

damage, respectively. To evaluate cardiac function, E-max and Tau (the time constant of early diastolic left ventricular pressure decay) before and after CPB were compared. Cardiac function values were input into a Sigma-5 (CardioDynamics Inc., Zoetermeer, the Netherlands), and analyzed with the conversion software PC (CardioDynamics Inc.). At the end of the experiment, a 1 cm<sup>2</sup> tissue specimen was collected from the free wall of the left ventricle around the first diagonal branch of the left anterior descending branch, and stained with hematoxylin and eosin (H-E) for histologic examination.

The experimental animals were divided into three groups: 1) the control group (seven pigs) that was left alone for 16 minutes after initiation of CPB, and was subjected to ascending aortic clamping and 40 minutes of cardioplegic arrest with a cardioplegia, followed by reperfusion, 2) the IPC group (seven pigs) that, after initiation of CPB, was subjected to 40 minutes of cardioplegic arrest with a myocardial protective solution, followed by reperfusion, and 3) the IPC+ATP group (seven pigs) that, after initiation of CPB, received 140  $\gamma$  of ATP (Adetphos, Kowa Company, Ltd., Tokyo, Japan) during IP, and was subjected to 40 minutes of cardioplegic arrest with cardioplegia, followed by reperfusion (Fig. 1). To evaluate cardiac function, the E-max before initiation of CPB was defined as 100%, recovery rates of cardiac contractile force after weaning from CPB were compared. To evaluate diastolic function, the level of Tau before initiation of CPB was defined as 1, and Tau levels after weaning from CPB were compared.

Results were expressed as means  $\pm$  standard deviations, and a one-way analysis of variance was used to test for differences in indicators measured at 4 timepoints in each



**Fig. 2.** The changes of blood adenosine during experiments.

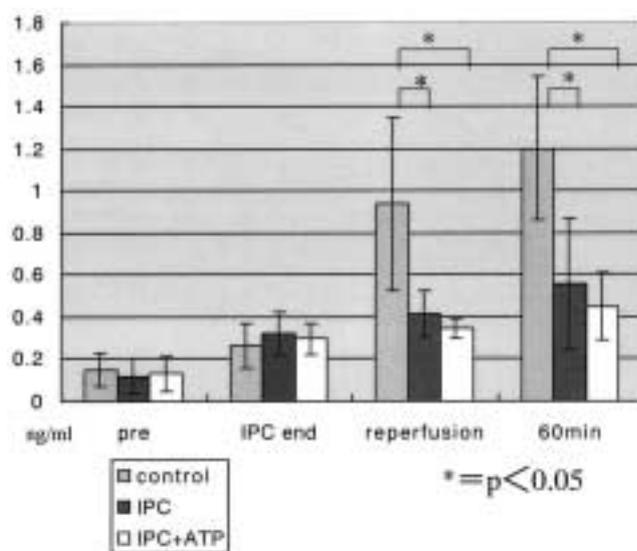
group and/or among the three groups. Significant differences among the three groups, if observed, were analyzed by the Scheff method of multiple comparisons, and  $p < 0.05$  was considered significant. All experiments were performed in accordance with the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science (Exp Anim 1987; 36: 285–8).

## Results

Rectal temperatures were maintainable at  $36.2 \pm 0.6^\circ\text{C}$ ,  $36.4 \pm 0.8^\circ\text{C}$ , and  $36.5 \pm 0.6^\circ\text{C}$  in the control, IPC, and IPC+ATP groups, respectively; and myocardial temperatures at  $16.6 \pm 0.6^\circ\text{C}$ ,  $16.4 \pm 0.4^\circ\text{C}$ , and  $16.3 \pm 0.5^\circ\text{C}$  in the control, IPC, and IPC+ATP groups, respectively. There were no inter-group differences. All animals in the three groups restarted heartbeat without defibrillation after declamping of the aorta, and were able to be weaned from CPB.

### Adenosine (Fig. 2)

There were no inter-group differences in the pre level of adenosine:  $19.9 \pm 5.6$  pmol/L (control group),  $20.4 \pm 7.8$  pmol/L (IPC group), and  $21.1 \pm 6.6$  pmol/L (IPC+ATP group). There were significant inter-group differences in the IPC-end level of adenosine, with the highest in the IPC+ATP group:  $11.3 \pm 4.5$  pmol/L (control group),  $89.7 \pm 28.5$  pmol/L (IPC group), and  $149.6 \pm 33.6$  pmol/L (IPC+ATP group). Compared with the control group, the remaining two groups showed significantly higher reperfusion and R-60-min levels of adenosine: reperfusion



**Fig. 3.** The changes of blood troponin-T during experiments.

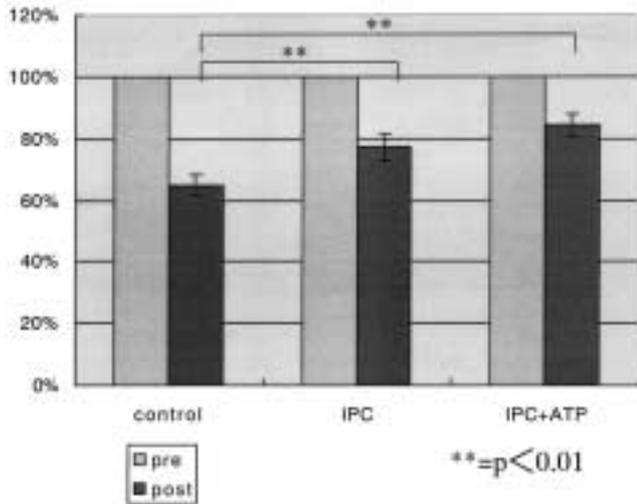
levels were  $15.8 \pm 7.1$  pmol/L (control group),  $93.6 \pm 24.6$  pmol/L (IPC group), and  $122.7 \pm 27.6$  pmol/L (IPC+ATP group); R-60-min levels were  $15.6 \pm 6.4$  pmol/L (control group),  $89.9 \pm 32.5$  pmol/L (IPC group), and  $107.8 \pm 23.4$  pmol/L (IPC+ATP group). Compared with the IPC group, the IPC+ATP group tended to have higher reperfusion and R-60-min levels of adenosine, showing no significant differences.

### Troponin-T (Fig. 3)

There were no inter-group differences in the pre level of troponin-T:  $0.15 \pm 0.08$  ng/ml (control group),  $0.12 \pm 0.08$  ng/ml (IPC group), and  $0.13 \pm 0.08$  ng/ml (IPC+ATP group). No clearly significant inter-group differences in the IPC-end level of troponin-T were found:  $0.26 \pm 0.10$  ng/ml (control group),  $0.32 \pm 0.10$  ng/ml (IPC group), and  $0.29 \pm 0.07$  ng/ml (IPC+ATP group). Compared with the control group, the remaining two groups showed significantly lower reperfusion and R-60-min levels of troponin-T: reperfusion levels were  $0.93 \pm 0.41$  ng/ml (control group),  $0.41 \pm 0.11$  ng/ml (IPC group), and  $0.34 \pm 0.04$  ng/ml (IPC+ATP group); and R-60-min levels were  $1.20 \pm 0.34$  ng/ml (control group),  $0.55 \pm 0.30$  ng/ml (IPC group), and  $0.44 \pm 0.16$  ng/ml (IPC+ATP group). Compared with the IPC group, the IPC+ATP group tended to have lower reperfusion and R-60-min levels of troponin-T, showing no significant differences.

### E-max (Fig. 4)

Compared with the control group, the remaining two groups showed significantly higher recovery rates of



**Fig. 4.** The changes of E-max (pre versus post experiment).

myocardial contractile force after weaning from CPB:  $65.0 \pm 3.4\%$  (control group),  $77.1 \pm 4.2\%$  (IPC group), and  $84.4 \pm 3.7\%$  (IPC+ATP group). Compared with the IPC group, the IPC+ATP group tended to have a higher recovery rate, showing no significant difference.

#### Tau (Fig. 5)

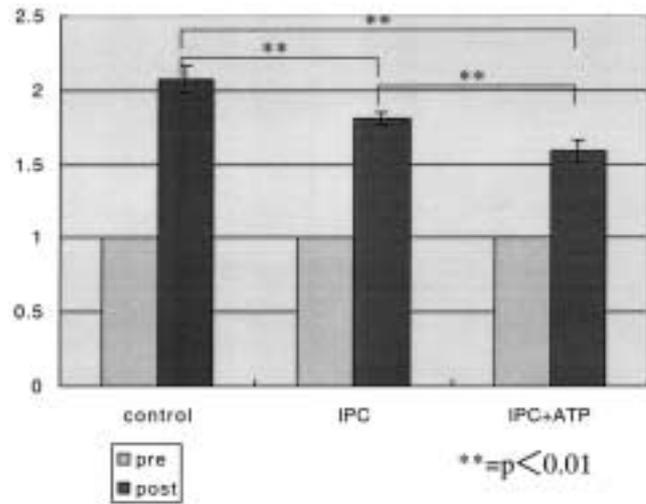
All three groups showed prolonged Tau as diastolic dysfunction after weaning from CPB:  $2.07 \pm 0.091$  (control group),  $1.81 \pm 0.045$  (IPC group), and  $1.59 \pm 0.075$  (IPC+ATP group). There were significant inter-group differences in the severity of diastolic dysfunction, with the IPC+ATP group being the least severely impaired and the control group being the most severely impaired.

#### Histopathological findings (Fig. 6)

H-E staining of tissue specimens after weaning from CPB revealed histological changes due to ischemia-reperfusion, with hypercontraction bands in all three groups (A-1, B, C-1). The control group showed widely distributed hypercontraction bands, with remarkable ischemic changes such as perinuclear vacuolar degeneration and waving degeneration of myofibrils (A-2). In the IPC group, the structure of myofibrils was well preserved, with no ischemic changes. In the IPC+ATP group, the structure and arrangement of myofibrils were well preserved (C-2).

#### Discussion

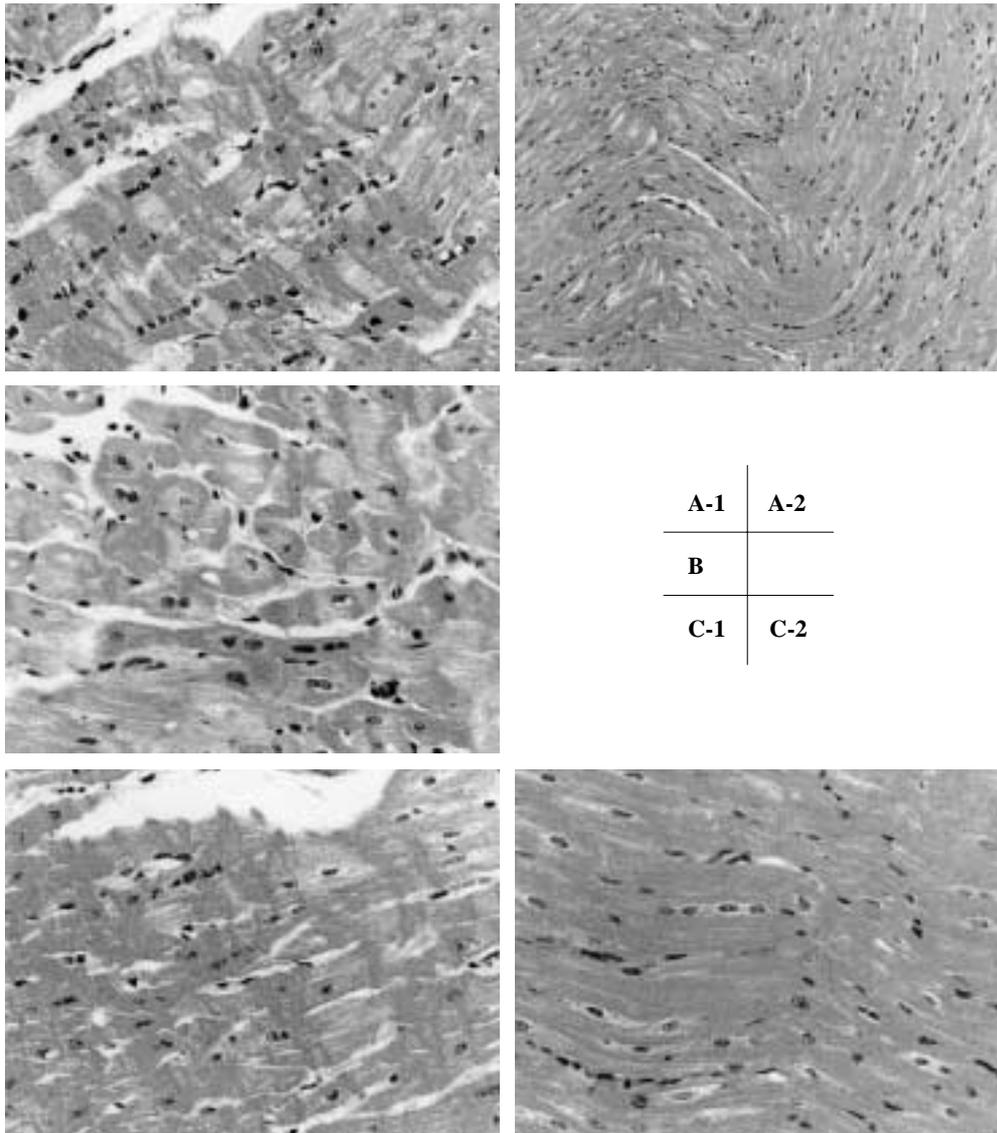
Since the myocardial protection method was introduced in the 1950s, it has played an important role in enabling



**Fig. 5.** The changes of Tau (pre versus post experiment).

cardiac surgery to be safely performed. Diastolic cardiac arrest is achieved by rapid infusion of high-concentration  $K^+$  in cardioplegia, and is considered to result from the disruption of excitation-contraction coupling ( $Ca^{2+}$  influx through  $Na^+/Ca^{2+}$  exchange and subsequent  $Ca^{2+}$  induced  $Ca^{2+}$  release) due to inhibition of the generation of the 0 phase of action potential (activation of potential-dependent  $Na^+$  channels) by depolarization of myocardial cell membranes (increase in  $K^+$  equilibrium potential). In other words, rapid cardiac arrest is considered to reduce cardiac contraction-associated ATP consumption, thereby delaying the occurrence of irreversible myocardial damage.<sup>2)</sup> On the other hand, IPC refers to a phenomena in which myocardial necrosis associated with prolonged ischemia-reperfusion is reduced by prior exposure to brief periods of ischemia. Murry et al.<sup>3)</sup> reported that in a dog model of myocardial infarction following 4 cycles of a 5-minute blockage of coronary blood flow and a subsequent 40-minute reperfusion, the size of myocardial infarction markedly decreased, and that prior exposure to brief periods of ischemia followed by prolonged ischemia delayed the reduction in myocardial ATP content, reduced myocardial oxygen consumption, preserved myocardial cell structure, and delayed ischemic cell death. Currently, the effects of IPC are widely recognized, and this infarct-reducing effect is observed irrespective of animal species: similar effects have been reported in rats,<sup>4,5)</sup> rabbits,<sup>6,7)</sup> dogs,<sup>3,8)</sup> and pigs,<sup>9,10)</sup> and acquisition of resistance to ischemia has been confirmed in isolated human myocardium.<sup>11)</sup>

The mechanism of IPC is thought to involve adenosine,  $\alpha$  receptors, G protein, protein kinase C (PKC),  $K_{ATP}$



**Fig. 6.** A-1: control group (H-E staining×200), A-2: control group (H-E staining×100)  
 B: IPC group (H-E staining×400)  
 C-1: IPC+ATP group (H-E staining×100), C-2: IPC+ATP group (H-E staining×200)

channels, Na-H pump, Ca channels, NO, and the glycolysis system. Since the administration of PKC inhibitors has been demonstrated to abolish the effect of IPC,<sup>12)</sup> the common mechanism of IPC is considered to involve the activation of PKC. Although the myocardial protective effect of IPC has also been demonstrated experimentally, studies have reported various results regarding its effect. Kolocassides et al.<sup>13)</sup> reported that there was no significant difference in the recovery rate of left ventricular pressure generated after reperfusion between a group undergoing IPC with a 3-minute ischemia and a 3-minute reperfusion plus a 35-minute cardioplegic arrest with St. Thomas' solution and a group undergoing cardioplegic

arrest without IPC, indicating that the use of IPC had no enhancing effect. They speculated that since both myocardial protective solutions and IPC protect myocardium by suppressing myocardial ATP consumption, the combined use of IPC and cardioplegic arrest does not enhance the myocardial protective effect. An experimental study by Hudspeth et al.<sup>14)</sup> reported that the combined use of pharmacologic preconditioning with an adenosine deaminase inhibitor and cardioplegic arrest resulted in good recovery of left ventricular function. Furthermore, Wu et al.<sup>15)</sup> clinically applied IPC to coronary bypass patients, and reported that the combined use of IPC and cardioplegic arrest produced good post-cardioplegic re-

covery of cardiac function. Thus, the cardioprotective effects of combined use of IPC and cardioplegic arrest have not been fully elucidated.

Since it has been reported that IPC has a memory effect,<sup>16)</sup> we expected that sufficient activation of PKC would have a persistent IPC effect after cardioplegic arrest in this study. Also, studies reported that the administration of adenosine early after ischemia-reperfusion reduced the extent of myocardial infarction, indicating that exogenous adenosine prevents reperfusion injury,<sup>17-19)</sup> which has attracted attention as a post-myocardial infarction therapy. However, since it has also been reported that exogenous adenosine administration alone does not prolong the memory effect of IPC,<sup>16)</sup> we anticipated that the administration of exogenous adenosine would enhance the cardioprotective effect during performance of IPC in this study. Yellon et al.<sup>11)</sup> reported that aortic cross clamping during CPB was one of the IPC methods, which prevented the decrease of myocardial ATP. In the present study, we also considered that global IPC might be effective as myocardial protection for the ischemic heart during cardioplegic arrest. Based on these observations and speculations, we compared the myocardial protective effects of combined use of IPC and cardioplegic arrest among the control, IPC and IPC+ATP groups. Since the administration of 140  $\mu$ g of the adenosine precursor ATP was reported to produce a good recovery of cardiac function after ischemia-reperfusion,<sup>20)</sup> we used the same dose of ATP.

The IPC-end levels of blood ATP were significantly higher in the IPC and IPC+ATP groups than in the control group. ATP, administered into the blood stream, is rapidly dephosphorylated and degraded to ADP, AMP, and eventually ATP. We speculated that the high IPC-end adenosine level in the IPC+ATP group was due to an increase in exogenous as well as endogenous adenosine. The post-cardioplegic adenosine level was also significantly higher in the IPC and IPC+ATP groups. This presumably resulted from an increase in adenosine due to the memory effect of IPC, which produced a cardioprotective effect. Although the IPC+ATP group tended to maintain a higher level of adenosine than the IPC group, there was no significant difference between the two groups, suggesting that exogenous adenosine does not provide a sufficient memory effect, or the memory effect attenuates over time.

Troponin-T increased after cardioplegic arrest in all three groups, and undoubtedly larger amounts of troponin-T were released in the control group, suggesting that the concomitant use of IPC can suppress myocardial damage.

Comparison of the myocardial contractile force indi-

cator E-max showed that the IPC and IPC+ATP groups achieved significantly better recovery of myocardial contractile force than the control group, but there was no difference between the IPC and IPC+ATP groups. Experiments on isolated canine papillary muscles have reported that adenosine has a ventricular contractile force-increasing effect following a brief contractile force-decreasing effect, that is, a slightly greater positive inotropic effect.<sup>21)</sup> Adenosine is metabolized to inosine, which also has been reported to have a positive inotropic effect on atrial and ventricular muscles.<sup>22,23)</sup> These, in concert, presumably contributed to the recovery of contractile force after weaning from CPB. We speculate that the absence of a significant difference in the level of adenosine after weaning from CPB between the IPC and IPC+ATP groups was associated with the absence of a clearly significant difference in E-max.

We measured Tau as an indicator of diastolic dysfunction, and found that the control group had the most severe diastolic dysfunction, the IPC group the next most severe dysfunction, and the IPC+ATP group the mildest dysfunction. During diastole, ATP is required for the dissociation of bound crossbridges and the incorporation of calcium into sarcoplasmic reticula, and myocardial relaxation is thought to be a process of energy consumption. There are some factors of diastolic function, such as cardiac compression, ventricular remodeling, or relaxation of myocytes. It is generally accepted that the delay of Tau indicates deterioration of myocardial relaxation. Thus, since the IPC+ATP group had the mildest diastolic dysfunction, we speculate that cardioplegic arrest suppressed intramyocardial ATP decrease, but we were not able to demonstrate it in this study. However, since the blood adenosine level was highest in the IPC+ATP group, and since the adenosine metabolite inosine has a potent atrial muscle-contracting effect, diastolic function may have been maintained.

Histologic examination also showed that the histologic structure of myocardium was relatively well preserved in the IPC and IPC+ATP groups, suggesting that the concomitant use of IPC enhances the myocardial protective effect of cardioplegic arrest. However, the administration of exogenous adenosine failed to enhance the cardioprotective effect in this study, suggesting the need for investigating the amount and timing of dosage of adenosine and the myocardial ATP content. Although we used a 40-minute period of ischemia in this study, the effect of IPC on longer myocardial ischemia needs to be investigated.

In general, heart surgery using cardioplegia carries a

high risk of severe postoperative complications such as low cardiac output syndrome and arrhythmias if cardioplegic arrest is prolonged. The results of this study suggest that the concomitant use of IPC reduces myocardial damage, allowing safer surgery.

## Conclusions

1. We experimentally investigated the effects of IPC on cardioplegic arrest and of premedication of adenosine.
2. The concomitant use of IPC enhanced the effect of a myocardial protective solution.
3. Although the administration of adenosine in conjunction with IPC suggested the possibility of enhancing the cardioprotective effect, it requires future studies.

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