

Myocardial Cyclic AMP Augmentation with High-Dose PDEIII Inhibitor in Terminal Warm Blood Cardioplegia

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Purpose: Phosphodiesterase (PDE) III inhibitors have been reported in various cellular protective activities via the cyclic adenosine monophosphate (cAMP) pathway. We investigated the effects of amrinone on ischemia/reperfusion injury and intracellular calcium (Ca^{2+}) handling if utilized as a component of terminal warm blood cardioplegia (TWBCP).

Methods: Anesthetized pig hearts were subjected to 90-min global ischemia with single-dose crystalloid cardioplegia, followed by 30-min reperfusion under cardiopulmonary bypass. The animals were divided into three groups according to the contents of TWBCP (n = 5 each): Control, no TWBCP; TWBCP, no additive; Amrinone, TWBCP with amrinone. The time course of cardiac function and biochemical samples were measured. Further, coronary perfusion and ventricular arrhythmia were evaluated during reperfusion.

Results: Cardiac function improved with amrinone. Specifically, the amrinone group showed an increase of myocardial cAMP (p <0.05) and a suppression of creatine kinase, troponin-T, and lipid peroxide after reperfusion (p <0.05); many cases also showed much improvement of coronary perfusion and spontaneous resuscitation after global ischemia.

Conclusion: Ischemia and/or reperfusion deplete myocardial cAMP, leading to impaired Ca^{2+} handling and further to cardiac dysfunction. High-dose PDEIII inhibitor in TWBCP may replenish myocardial cAMP and promote rapid and sustained cardiac functional recovery with various cellular protective effects after open-heart surgery. (*Ann Thorac Cardiovasc Surg* 2009; 15: 311–317)

Key words: blood cardioplegia, cyclic adenosine monophosphate, ischemia/reperfusion injury, phosphodiesterase III inhibitor

Introduction

The advances in heart surgery and the dramatic improvements in outcomes seen in recent years have in part been the result of a better understanding of ischemia/reperfusion (I/R) injury and advances in myocardial protection.¹⁾

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However, acute myocardial dysfunction still threatens the outcome of complex cases that require longer ischemic times. Amrinone, one of the phosphodiesterase (PDE) III inhibitors, inhibits PDEIII, which is localized mainly in both myocardium and vascular smooth muscle, increasing intracellular cyclic adenosine monophosphate (cAMP) levels.²⁾ cAMP potentiates cardiac contraction and vasodilatation with its Ca^{2+} handling, and it is also a second messenger of intracellular transmission with various cellular protective effects. We speculated that this compound could confer protection against I/R injury and correct intracellular Ca^{2+} handling during cardiac I/R. The purpose of this study was to investigate the effects of amrinone on prolonged cardiac ischemia by adding it to terminal warm

blood cardioplegia (TWBCP).

Materials and Methods

All animals received humane care in compliance with the *Principles of Laboratory Animal Care*, formulated by the Institute of Laboratory Animal Resources, and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised in 1996.

Animal preparation

Fifteen White-Landrace-Duroc pigs (one month old, 10–12 kg) were premedicated (Ketamine 15 mg/kg and diazepam 0.5 mg/kg, intramuscularly) and anesthetized with inhaled sevoflurane (1.0%–1.5%). Support with a volume-controlled ventilator (ACE-300, ACOMA, Japan) was started after endotracheal intubation. The femoral artery and vein were cannulated, the animals were kept on arterial blood oxygen and carbon dioxide tension, and the pH values were within the normal range. After median sternotomy, an apical solid-state pressure transducer-tipped catheter (MPC-500, Millar Instruments Inc., Houston, TX) was inserted to monitor left ventricular (LV) pressure. LV dimensions were measured with endocardially placed ultrasonic microtransducer crystals (Sonometrics Corp., London, ON, Canada). Two pairs of crystals were positioned across the minor and major cardiac axes. LV volume was assessed by an ellipsoid-based formula. Pressure volume loops were recorded digitally. After systemic heparinization (300 U/kg), extracorporeal circulation with a membrane oxygenator (CapiioxSX SX10, Terumo Corp., Japan) and an extracorporeal pump (Advanced Perfusion System 1, Terumo Corp., Japan) was achieved using a 12 F aortic cannula through the carotid artery and a 24 F right-angle cannula placed into the right atrium. Oxygen tension was kept at 300 mmHg, and aortic pressure was kept at 50–70 mmHg by adjusting the flow to keep a mixed venous oxygen saturation of approximately 70% at normothermia. The hematocrit was kept at 25%–30% with donor blood and lactated Ringer's solution. Electrolytes and pH were kept at normal levels. The coronary sinus was cannulated for blood sampling, and both the LV and pulmonary arteries were vented.

Experimental protocol

Cardiac arrest was achieved with a single dose of cold crystalloid cardioplegic solution (Miotector®, Mochida

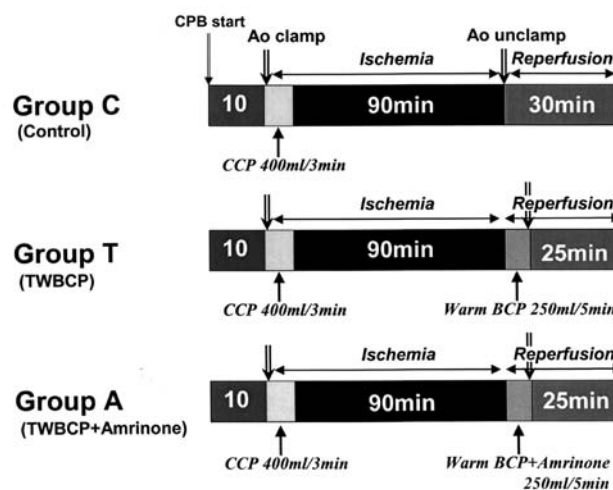


Fig. 1. Experimental protocol.

CPB, cardiopulmonary bypass; CCP, crystalloid cardioplegia; TWBCP, terminal warm blood cardioplegia; BCP, blood cardioplegia.

Pharmaceutical Co. Ltd., Japan), and TWBCP (blood: crystalloid = 4:1) based on Buckberg's method¹⁾ was performed for 5 min at 50 ml/min following 90 min of normothermic cardiac arrest. The animals were randomly divided into three groups ($n = 5$ each) according to the contents of TWBCP: Control (C), no TWBCP; TWBCP (T), no additive; Amrinone (A), in which amrinone was added to TWBCP and the concentration was regulated to 15 $\mu\text{g}/\text{ml}$ in the solution. Thirty minutes after reperfusion, the animals were weaned from cardiopulmonary bypass (CPB) without any inotropes. The experimental protocol is shown in Fig. 1.

Measurement

Left ventricular performance

LV pressure received from a Millar catheter and ultrasonic signals from sonomicrometer crystals placed on the anteroposterior LV epicardial surface were amplified and digitalized to inscribe LV pressure volume loops. A series of pressure volume loops was generated under varying loading conditions by transient occlusion of the inferior vena cava during a 10-sec absence of ventilator assistance. Measurements were made before CPB as control and 10 min after CPB was discontinued. End-systolic elastance (Ees) and a time constant of isovolumetric relaxation (Tau) were analyzed by a SonoSOFT recording (Sonometrics, London, ON, Canada) as indexes of LV contractility and diastolic function, respectively. All data at postbypass were expressed as a percent recovery of prebypass control values.

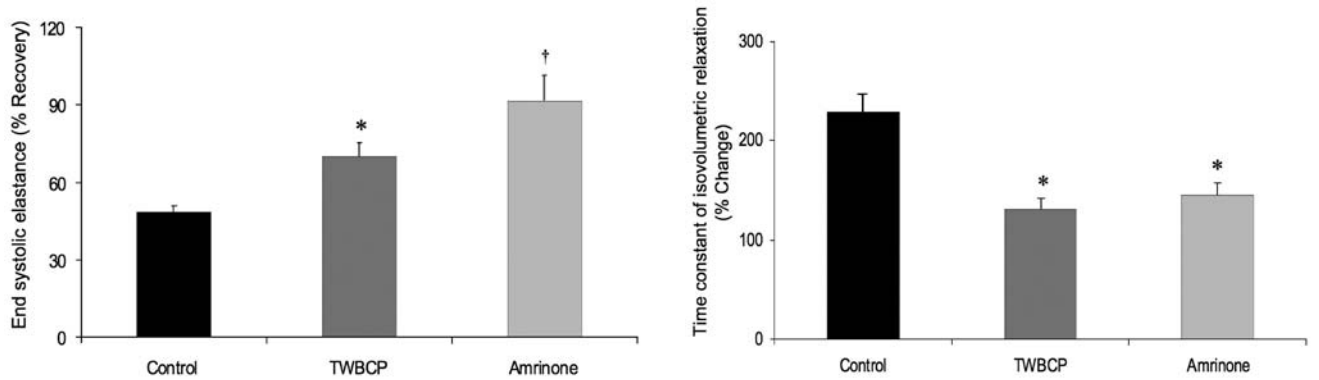


Fig. 2. Left ventricular performance evaluated by pressure volume loops.

A: The % recovery of end-systolic elastance (Ees); index of left ventricular contractility.

B: The % change of time constant of isovolumetric relaxation (Tau); index of left ventricular relaxation.

(*p < 0.05 vs. control, †p < 0.05 vs. TWBCP)

TWBCP, terminal warm blood cardioplegia.

A | B

Biochemical analyses

Coronary sinus blood samples were taken before CPB and 0, 10, 30, and 60 min after aortic unclamping. Myocardial injury was determined by measuring creatine kinase (CK) with an ultraviolet radiation method and by the electrochemiluminescence immunoassay (ECL-IA) method for troponin-T. As a marker of antioxidant reserve capacity, one of O₂ metabolites, lipid peroxide (LPO) levels were measured by the hemoglobin methylene blue method. A small piece of right ventricle free-wall muscle was taken from all animals before CPB and 0 and 30 min after reperfusion. The myocardial cAMP level in the homogenized tissue was then measured by the radioimmunoassay method and expressed as pmol/mg wet weight.

Coronary vascular response

All animals except for control were evaluated by coronary flow with perfusion pressure under the same conditions. The coronary perfusion pressure was measured with a cardioplegia route just before the end of TWBCP under a constant flow of 50 ml/min.

Ventricular arrhythmia

We measured total Joules of cardioversion performed after reperfusion because the energy levels of the animals had to be resuscitated against ventricular arrhythmia. All animals were given lidocaine (1 mg/kg) intravenously just after aortic unclamping.

Statistical analysis

All data were expressed as mean \pm standard error of the mean. Statistical analysis of variance (ANOVA) was used for intergroup comparisons, and the paired Student's *t*

test was for comparison of variables within experimental groups. All data were analyzed on the software StatView Version 5.0 (Abacus Concepts Inc., Berkeley, CA). Changes within and between groups were considered statistically significant when the *p* value was less than 0.05.

Results

Left ventricular performance

The percentage of recovery or change from baseline values is shown in Fig. 2, and regarding LV contraction (Ees), group A was 91.8% \pm 10.6% and showed significant recovery much more than the others (*p* < 0.05 vs. C; 48.6% \pm 2.2% and T; 69.9% \pm 5.5%). On the other hand, in the assessment of LV relaxation (Tau), groups T (131% \pm 10%) and A (145% \pm 12%) improved (*p* < 0.05 vs. C; 228% \pm 19%), and no clear impairment in the amrinone group was observed.

Plasma biochemical activity

All data are shown as the percentage of baseline values in Fig. 3. Amrinone suppressed both CK and troponin-T after reperfusion; In particular, both values at 60 min after reperfusion were significantly suppressed as 177% \pm 22% and 555% \pm 69%, respectively (*p* < 0.05 vs. C; 323% \pm 32% and 1,150% \pm 110%, T; and 255% \pm 15% and 813% \pm 102%). With regard to LPO, the blood level had been sufficiently controlled from the early stage during reperfusion in the amrinone group, and the values were significantly lower than in the other groups (*p* < 0.05 vs. C and T).

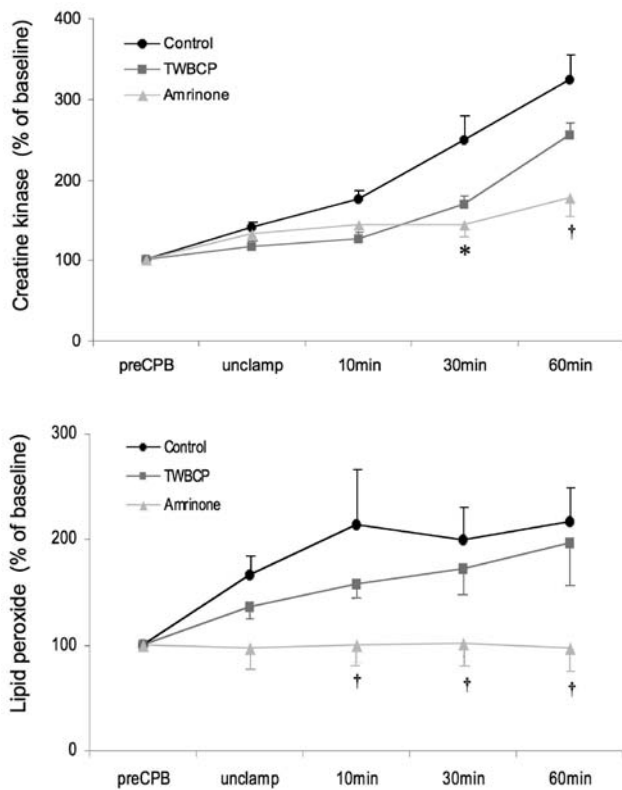


Fig. 3. Time course of serum levels of (A) creatine kinase, (B) troponin-T, and (C) lipid peroxide during ischemia/reperfusion. (*p < 0.05 vs. control, †p < 0.05 vs. TWBCP) TWBCP, terminal warm blood cardioplegia.

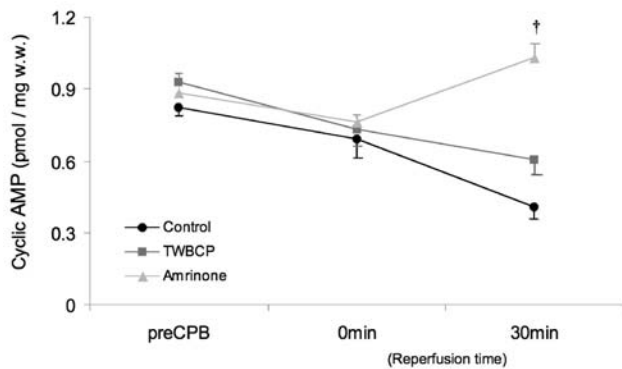
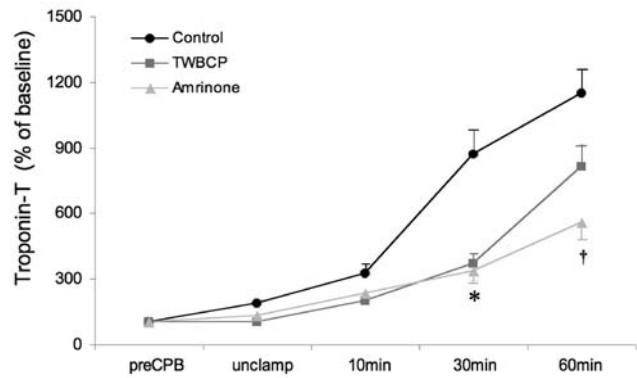


Fig. 4. Time course of myocardial cyclic AMP level during ischemia/reperfusion. Reperfusion time 0 min = the end of ischemia. (†p < 0.05 vs. TWBCP) TWBCP, terminal warm blood cardioplegia.

Myocardial cyclic AMP levels

The change of myocardial cAMP level is shown in Fig. 4. cAMP decreased during ischemia in all groups, and the condition was continued even after reperfusion in groups C and T, but only the amrinone group showed a significant



| | |
|---|---|
| A | B |
| C | |

increase after reperfusion. cAMP value at 30 min after reperfusion was 1.03 ± 0.06 pmol/mg wet weight in the amrinone group (p < 0.05 vs. C; 0.41 ± 0.05 pmol/mg wet weight, vs. T; 0.60 ± 0.05 pmol/mg wet weight).

Coronary vascular response

Coronary perfusion pressures at the end of TWBCP were 25.2 ± 4.3 mmHg in group T and 10.2 ± 1.9 mmHg in group A, respectively (Fig. 5). Amrinone significantly decreased perfusion pressure because of low resistance, leading to smooth coronary flow (p < 0.05 vs. T).

Ventricular arrhythmia

Most ventricular arrhythmias in this study were represented by ventricular fibrillation or ventricular tachycardia, and the total Joules of cardioversion until resuscitation were 51.4 ± 10.1 J (C), 39.1 ± 8.2 J (T), and 21.9 ± 4.2 J (A), respectively (Fig. 6). In the amrinone group, three of five cases resuscitated spontaneously during reperfusion, and the total electrical discharge was smaller than in other groups (p < 0.05 vs. C, T).

Blood concentration of amrinone

The change of blood concentration of amrinone after TWBCP was assessed in our preliminary study, and the data were shown in Fig. 7. The initial concentration of amrinone in TWBCP was $15,025 \pm 776$ ng/ml, and the whole blood concentration of amrinone immediately decreased to 232 ± 84 ng/ml after unclamping, with no reascension. None of them ever reached a value greater

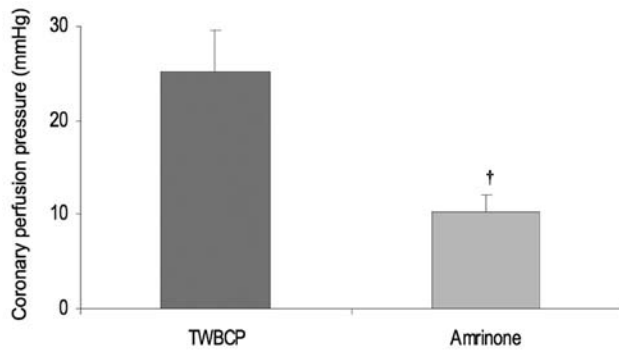


Fig. 5. Coronary perfusion pressure at the end of TWBCP. ($\dagger p < 0.05$ vs. TWBCP)
TWBCP, terminal warm blood cardioplegia.

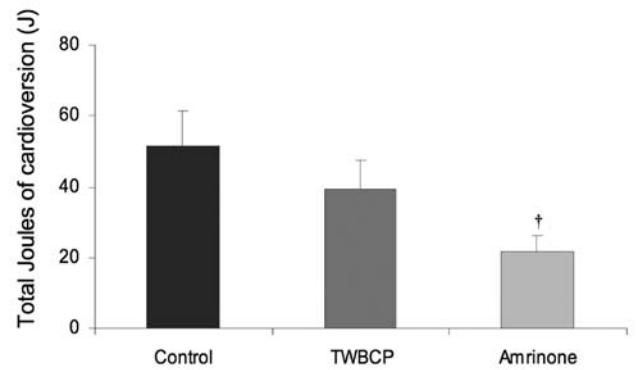


Fig. 6. Total joules of cardioversion for resuscitation. ($\dagger p < 0.05$ vs. TWBCP)
TWBCP, terminal warm blood cardioplegia.

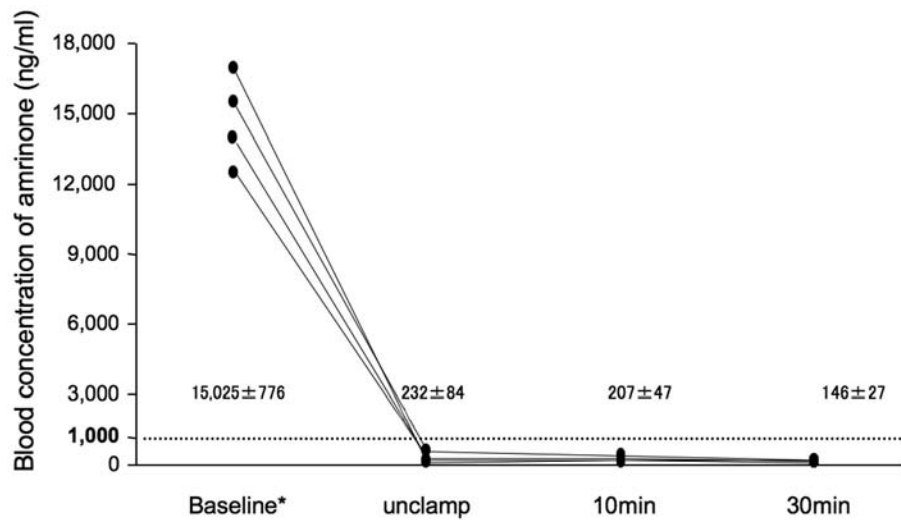


Fig. 7. Blood concentration of amrinone.
*Baseline is a concentration of amrinone in TWBCP, and others are in whole blood taken from coronary sinus. A dotted line indicates a minimum limit of pharmacological effects against cardiovascular.

than 1,000 ng/ml, which is considered to be the minimum limit of pharmacological effects against heart and vessels.²⁾

Discussion

The depletion of substance and adenosine triphosphate (ATP) in myocardium during ischemia is one of the major issues to promote cardiac function after open-heart surgery. Not only a supply of substance, but also an early replenishment of myocardial cAMP with an early correction of Ca²⁺ handling in the myocyte after ischemia should be important considerations in early and sustained recovery of the heart. An interrelation between myocardial cAMP concentration and cardiac function with amrinone was

described by a previous *in vitro* study.²⁾ Furthermore, Shahid and Rodger³⁾ reported that cAMP was one of the essential factors of Ca²⁺ handling at reperfusion, and it inhibited Ca²⁺ overload; Ishikawa et al.⁴⁾ showed the reduction of tissue injury with a replenishment of cAMP, using amrinone in dog liver, in which cAMP had been depleted during I/R. These reports would be the evidence to support our hypothesis. PDEIII inhibitors increase myocardial cAMP, which controls Ca²⁺ handling through the sarcoplasmic reticulum (SR) to promote myocyte activity, and they work independently of ATP without the down-regulation mechanisms common to other inotrops working via the β -receptor.^{2,3,5)} Moreover, the drugs have been reported to have various effects against harmful

phenomena caused by I/R.⁶⁻¹¹ Accordingly, we postulated that myocardial protection with a PDEIII inhibitor should be more effective to minimize I/R injury, acting in specific ways with many compounding effects. The rationale for using amrinone in the other PDEIII inhibitors is based on its low inotropic effect¹² of avoiding hyperconstriction and an outstanding potency as a free radical scavenger.¹⁰ And it has more rapid displacement into myocardium and longer duration in each pharmacokinetic comparison. We also showed the effects in the inhibition of LPO production and the elevation and duration of myocardial cAMP level after reperfusion in our unique procedure. The dose of amrinone used in this study was chosen to avoid the influence on cardiac function of the residue in blood after TWBCP, following our preliminary study in Fig. 7, and also the data of the relationship between the blood concentration and loading dose of amrinone described by Lawless et al.¹³ Further, we simultaneously applied the most effective concentration of amrinone to scavenge reactive oxygen species in reference to a previous *in vitro* study.¹⁰ According to some reports,^{12,14} PDEIII inhibitor increases cardiovascular effects in a dose-dependent fashion, and there is a major difference of the efficacies among PDEIII inhibitors of the same kind. In fact, regarding LV relaxation, unlike our results, previous investigators^{15,16} showed positive lusitropic effects with milrinone or highly concentrated amrinone. In view of these, we must carry on with the dose-related effects and the comparative studies with other PDEIII inhibitors in further research.

A relationship between Ca²⁺ overload and myocardial cAMP increased by drugs remains controversial. Some reports^{17,18} have documented the risks of pretreatment on an ischemic heart with catecholamine, but in this study, amrinone caused no negative effects associated with Ca²⁺ overload, such as fatal arrhythmia or diastolic dysfunction, though myocardial cAMP increased significantly. PDEIII inhibitor may enable enough accumulation of myocardial cAMP, even under cardiac arrest, because it works regardless of the ATP storage and the down regulation. And selective inhibition of PDEIII may also induce a localized cAMP accumulation on SR followed by a sustained high performance of SR as the main regulator of intracellular Ca²⁺ concentration, as Yano et al.¹⁵ reported. On the basis of the above, if the voltage-dependent Ca²⁺ channel and the Na⁺-Ca²⁺ exchanger as the main intracellular influx gates of Ca²⁺ would both be completely blocked by a low electrical potential and an acidosis during cardiac arrest, this modified TWBCP should enable us to set up and correct intracellular Ca²⁺

handling with the replenished cAMP and the stored ATP to react against excessive Ca²⁺ influx following the early stages of reperfusion.

There are many sources of oxidant generation during I/R,¹⁹ and that is one reason why clinical trials using antioxidants have failed to show clear benefits after the ischemic events, but PDEIII inhibitor has been reported to affect its potency as an antioxidant in multiple aspects, following the increase of cellular cAMP level. In fact, it plays a role not only in controlling inflammatory reactants of cytokine or neutrophil,^{8,11} but also in directly inhibiting activated oxygen radicals.^{9,10} We also showed strong and prompt efficacy of amrinone as a free radical scavenger, as documented by the inhibition of LPO from early stages, which resulted in a suppression of both CK and troponin-T during the late stages.

Improved coronary perfusion with low vascular resistance and spontaneous recovery in regard to normal sinus rhythm have been considered as signs of good myocardial protection after I/R.^{20,21} In our observation, amrinone prevented coronary flow obstruction and ventricular arrhythmia after I/R. This has been reported in previous animal studies,^{22,23} but we could demonstrate using amrinone safely and effectively with selective coronary administration simulating open-heart surgery. Moreover this procedure is simple and easy, and it will be a surgical option in cases of accidental long-time ischemia or very low cardiac function.

In summary, high-dose PDEIII inhibitor with TWBCP is expected to minimize I/R injuries with a replenishment of myocardial cAMP and other various cellular protective effects. Especially in cases of lengthy ischemic time or low cardiac function, myocardial cAMP augmentation with PDEIII inhibitor may play an important role in early and sustained cardiac recovery after open-heart surgery.

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