ORIGINAL ARTICLE

Reversal of oxidant-mediated biochemical injury and prompt functional recovery after prolonged single-dose crystalloid cardioplegic arrest in the infantile piglet heart by terminal warm-blood cardioplegia supplemented with phosphodiesterase III inhibitor

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Abstract
Purpose. The benefit of terminal blood cardioplegia (TWBCP) is insufficient after prolonged ischemia associated with inevitable oxidant-mediated injury by this modality alone. We tested the effects of TWBCP supplemented with high-dose olprinone, which is a phosphodiesterase III inhibitor, a clinically available compound with the potential to reduce oxidant stress and calcium overload. We evaluated the effects with respect to avoiding oxidant-mediated myocardial reperfusion injury and prompt functional recovery after prolonged single-dose crystalloid cardioplegic arrest in a infantile piglet cardiopulmonary bypass (CPB) model.
Methods. Fifteen piglets were subjected to 90 min of cardioplegic arrest on CPB, followed by 30 min of reperfusion. In group I, uncontrolled reperfusion was applied without receiving TWBCP; in group II, TWBCP was given; in group III, TWBCP was supplemented with olprinone (3 μg/ml). Myocardial performance was evaluated before and after CPB by a left ventricular (LV) function curve and pressure–volume loop analyses. Biochemical injury was determined by measurements of troponin-T and lipid peroxide (LPO) in coronary sinus blood.
Results. Group III showed significant LV performance recovery (group I, 26.5% ± 5.1%; group II, 42.9% ± 10.8%; group III, 81.9% ± 24.5%, $P < 0.01$ vs. groups I and II), associated with significant reduction of troponin-T and LPO at the reperfusion phase. No piglets in group III needed electrical cardioversion.
Conclusion. We concluded that TWBCP with olprinone reduces myocardial reperfusion injury by reducing oxidant-mediated lipid peroxidation, and it accelerates prompt and persistent LV functional recovery with suppression of reperfusion arrhythmia.

Introduction

Despite recent progress in myocardial protective techniques, postoperative myocardial dysfunction attributable to ischemic/reperfusion damage remains a major issue, contributing significantly to mortality and morbidity in settings involving increasingly complex surgical procedures and longer ischemic duration. Furthermore, current myocardial protection techniques are not always optimal, particularly for pediatric patients compromised by complex heart defects.[1]

Terminal blood cardioplegia (TWBCP) has been shown to accelerate myocardial metabolic recovery characterized by a more rapid shift to aerobic metabolism and better preservation of tissue adenosine triphosphatase and glycogen concentration after ischemia.[2]

However, the effects of TWBCP may be insufficient after prolonged ischemia associated with inevitable myocardial oxidants-mediated injury by this modality alone.

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Weisel et al.[3] clinically demonstrated the involvement of free-radical biochemical injury in myocardial dysfunction after the routine blood cardioplegic strategy with TWBCP. It manifested as increased myocardial release of conjugated dienes (chemical signature of free-radical-mediated lipid peroxidation) and decreased myocardial concentration of α-tocopherol (endogenous antioxidants). Weisel et al. therefore suggested the need for antioxidant therapy. To date, however, no clinically feasible modality has been adopted routinely to reduce oxidant-related injury. Thus, a safe, reliable therapeutic modality that can be used to avoid potential oxidant-mediated damage is needed. It should be available even at the onset of reperfusion, when ischemic intervals are unexpectedly prolonged, or myocardial protection is implemented inappropriately.

Phosphodiesterase (PDE) III inhibitors, used extensively to treat heart failure for their vasodilatory and inotropic effects,[4] have also been shown to confer cardioprotective effects against ischemia and reperfusion. The mechanism is through various cellular actions with the potential to reduce oxidant stress and calcium overload either directly or via enhanced intracellular cyclic adenosine monophosphate (cAMP).[5–9] Its potential benefits against reperfusion-induced oxidant-related injury have been experimentally shown in various organs, including the liver,[10] lungs,[11] kidneys,[12] and heart.[13,14] However, the benefits of this drug, when administered at the early reperfusion phase, has yet to be demonstrated because of potential concerns involving calcium overload by this drug as this is the most vulnerable time for intracellular calcium overload.[15]

We tested the effects of TWBCP supplemented with high-dose olprinone, a PDE III inhibitor, which is a clinically available compound with the potential to reduce oxidant stress and calcium overload. We evaluated the effects with respect to avoiding oxidant-mediated myocardial reperfusion injury and prompt functional recovery after prolonged global ischemia in an infantile piglet cardiopulmonary bypass (CPB) model. We sought to simulate the clinical conditions of prolonged ischemia arising from inappropriately implemented myocardial protection.

Material and methods

Experimental model

We used fifteen 4-week-old Large White-Landrace-Duroc piglets weighing 10–12 kg. Anesthesia was initiated with ketamine hydrochloride 4 mg/kg given intravenously and maintained by sevoflurane (1.0%–

1.5%). After endotracheal intubation, respiration was controlled by a ventilator (Anesthesia Apparatus PH-5F; Acoma, Tokyo, Japan). Catheters were placed in the left femoral artery and vein for continuous monitoring of the systemic blood and central venous pressures and for withdrawing blood or administering drugs and fluids. Electrocardiographic leads were attached to measure heart rate and monitor for ventricular arrhythmias.

After median sternotomy, an apical solid-state pressure transducer-tipped catheter (MPC-500; Millar, Houston, TX, USA) was inserted to monitor left ventricular (LV) pressure. Two pairs of crystals were positioned across the minor and major cardiac axes. LV dimensions were measured with endocardially placed ultrasonic microtransducer crystals (Sonometrics, London, Ontario, Canada). The LV volume was assessed by an ellipsoid-based formula. Pressure–volume loops were recorded digitally.

After systemic heparinization (300 IU/kg), we achieved extracorporeal circulation with a membrane oxygenator (CapioxSX SX10; Terumo, Tokyo, Japan) and an extracorporeal pump (Advanced Perfusion System I; Terumo) using a 12F arterial perfusion cannula placed in the prepared right common carotid artery and a right-angle venous cannula placed in the right atrium. The left atrium was vented and the left atrial pressure measured with a 12F catheter inserted via the left atrial appendage. The pulmonary artery was vented with a 12F catheter inserted via the main pulmonary artery. The coronary sinus was cannulated with a catheter for blood sampling.

The total priming volume of the bypass circuit was 400 ml. The bypass circuit was primed with heparinized homologous whole blood (150 ml) drawn from a donor pig, acetated Ringer's solution, hydroxyethylated starch, D-mannitol, and sodium bicarbonate followed by hemoconcentration with extracorporeal ultrafiltration to achieve an intrabypass hematocrit of 25%–30%. Oxygen tension was kept at 300 mmHg. Aortic pressure was kept at 50–70 mmHg by adjusting flow to maintain a mixed venous oxygen saturation of approximately 70% at normothermia. Electrolytes and pH were kept at normal levels.

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 (published by the National Academy Press, revised 1996).

Experimental groups

The animals were assigned to one of three groups (five animals per group) based on the reperfusion mode.

Group I had uncontrolled reperfusion, applied by simply unclamping the aorta without TWBCP. Group II had controlled reperfusion, applied by TWBCP. Group III had TWBCP supplemented with olprinone 3 µg/ml. The dosage of olprinone in TWBCP in this experiment was determined to provide the antioxidant effects previously described.[5,16]

Experimental protocol

Following animal preparation and instrumentation, baseline control measurements of all parameters were obtained. An aortic cross-clamp was applied 10 min after starting CPB under normothermia. Cardiac arrest was achieved with a single dose of cold (4°C) St Thomas Hospital II solution (Miotector; Mochida Corp, Japan) at a pressure of 50–80 mmHg. The heart was then subjected to 90 min of global ischemia with topical pericardial cooling (saline ice slush) and without any additional cardioplegic solution. After 90 min of ischemia, 250 ml of warm (37°C) hypocalcemic (0.4–0.5 mmol/l) blood cardioplegia solution (4:1, blood/crystalloid) was infused for 5 min at 50 ml/min, after which the aortic cross-clamp was released.

The ingredients of the crystalloid solution for TWBCP and the final blood cardioplegia solution delivered are shown in Table 1. The administration of olprinone 3 µg/ml in group III did not alter the pH and electrolyte concentration of BCP. Thirty minutes after reperfusion, animals were weaned from CPB without inotropes and followed for 30 min after termination of CPB.

Myocardial performance

Global cardiac performance from LV function curve

The integrated LV performance was evaluated by LV function curve analysis. Hemodynamic measurements were performed before CPB was initiated (control); during the early reperfusion phase—just after CPB was discontinued (30 min after reperfusion); and during the late reperfusion phase—30 min after termination of CPB (60 min after reperfusion). Cardiac output (CO) was measured with a transit time flow meter (T201; Transonic System, Ithaca, NY, USA) placed around the ascending aorta while the left atrial pressure (LAP) was altered by serial volume loading from the extracorporeal circuit to assess the LV function curve (LAP vs. LV stroke work index). The LV stroke work index (LVSWI) was calculated by the following equation:

$$LVSWI\,(g.m/kg) = (MAP - LAP) \times CO\,(ml/min) \times \\ 0.0135/[HR \times body\ weight\,(kg)]$$

where MAP is the mean aortic pressure, LAP is the mean left atrial pressure, and HR is the heart rate.

Left ventricular function curves were then inscribed by plotting X (LAP) against Y (LVSWI) with three or more data points by serial volume loading to alter the LAP from 3 mmHg to 12 mmHg. Straight regression lines were constructed, and the trapezoid area underneath the regression lines between 3 and 12 mmHg of LAP was measured. LV performance recovery after CPB was assessed by the percent of the trapezoid area of the pre-CPB control.

LV pressure–area loop

We measured LV pressure volume loops with sonomicrometry crystals and Millar catheters. These data were recorded by rapid transient occlusion of the inferior vena cava during a 7-s period of apnea, at control conditions before CPB, and 30 and 60 min after reperfusion. The LV systolic and diastolic performances were described as end-systolic elastance (Ees)[17] and the time constant of isovolumic relaxation (tau),[18] respectively, analyzed by a computer program (SonoSOFT Version 3.4.30 RC5; Sonometrics, London, ONT, Canada). LV performance after CPB was assessed by percent recovery of the pre-CPB control.

Arrhythmia

If a heart fibrillated within 3 min after aortic declamping, it was defibrillated once with 20 J of electrical cardioversion. The total quantity of electrical cardioversion after reperfusion was recorded.

Biochemical measurements

Coronary venous blood samples were drawn from the coronary sinus before CPB and at 0, 10, 30, and 60 min

Table 1 Ingredients of crystalloid solution for TWBCP and the final blood cardioplegia delivered

Crystalloid solution for TWBCP		Final data for blood cardioplegia	
Solution	Content	Parameter	Concentration
Miotector (A/B)	400.0 ml	pH	7.361 ± 0.092
KCl (1 mEq/ml)	22.0 ml	Na (mmol/l)	131 ± 5.2
CPD	100.0 ml	K (mmol/l)	16.3 ± 2.2
NaHCO₃	25.0 ml	Ca (mmol/l)	0.54 ± 0.03
Olprinone (1 mg/ml)	1.2 ml[a]	Hb (g/dl)	7.8 ± 2.2

TWBCP, terminal blood cardioplegia; Hb, hemoglobin; CPD, Citrate Phosphate Dextrose solution

[a] Group III

after aortic unclamping. Myocardial cellular injury was determined by measuring troponin-T by the ECL-IA method. We measured lipid peroxide (LPO) levels by the hemoglobin methylene blue method. LPO was considered a marker of oxidant-mediated lipid peroxidation.

Olprinone concentration

We measured olprinone concentrations during TWBCP and drew coronary venous blood samples from the coronary sinus at 0, 10, 30, and 60 min after aortic unclamping.

We measured myocardial tissue olprinone concentrations (in ng eq./g) in group III 60 min after aortic unclamping at the end of the protocol by radioimmunoassay method.

Myocardial cyclic AMP content

Transmural samples of the left ventricular free wall were taken at the end of protocol. These samples were frozen and stored at −80°C until assay.

Then cAMP content (pmol/mg wet weight) of tissue homogenates was measured by the radioimmunoassay method.

Statistical analysis

All data are expressed as means ± SD. One-way analysis of variance was used to determine statistical significance among groups. When we obtained a significant F ratio, we undertook further analysis with Scheffe's F post-hoc test used to identify significant differences between groups. Statistical significance was acceptable to a level of $P < 0.05$.

Results

Myocardial performance

Hemodynamic parameters and LV function curve analysis

Hemodynamic data (before bypass control and 30 and 60 min after reperfusion) are summarized in Table 2. There were no significant differences in any of the parameters between the three groups before CPB. After reperfusion, there were significant increases in aortic pressure and cardiac output in group III compared to groups I and II, whereas no change was noted in systemic venous resistance in groups II and III.

Table 2 Hemodynamic data for pre-CPB (control) 30 and 60 min after reperfusion

Parameter	Group I	Group II	Group III
Heart rate (beat/min)			
Control	117.4 ± 15.9	99.9 ± 8.3	116.6 ± 8.6
30 min after reperfusion	143.6 ± 16.5	135.0 ± 7.2	140.9 ± 9.3
60 min after reperfusion		131.7 ± 14.2	141.9 ± 7.3
Systolic aortic pressure (mmHg)			
Control	88.2 ± 18.0	89.8 ± 16.5	85.8 ± 4.5
30 min after reperfusion	55.4 ± 18.6	82.7 ± 10.7*	91.8 ± 8.0*
60 min after reperfusion		71.8 ± 5.8	90.0 ± 7.0#
Left atrial pressure			
Control	5.0 ± 1.1	4.5 ± 2.3	4.6 ± 0.9
30 min after reperfusion	8.3 ± 2.2	7.0 ± 2.6	6.6 ± 2.9
60 min after reperfusion		7.1 ± 1.8	5.6 ± 2.1
Cardiac output (l/min)			
Control	0.885 ± 0.170	0.871 ± 0.138	0.860 ± 0.135
30 min after reperfusion	0.492 ± 0.109	0.673 ± 0.086*	0.913 ± 0.152*#
60 min after reperfusion		0.670 ± 0.150	0.819 ± 0.211 #
Systemic venous resistance (SVR)[a]			
Control	63.7 ± 8.3	66.7 ± 23.3	66.6 ± 6.0
30 min after reperfusion	70.6 ± 17.3	102.4 ± 29.0	83.2 ± 15.5
60 min after reperfusion		98.4 ± 20.9	98.4 ± 22.3

The results are the mean ± SD

[a] SVR = mean AP-CVP (aortic pressure/central venous pressure)/CO

* $P < 0.01$ vs. group I
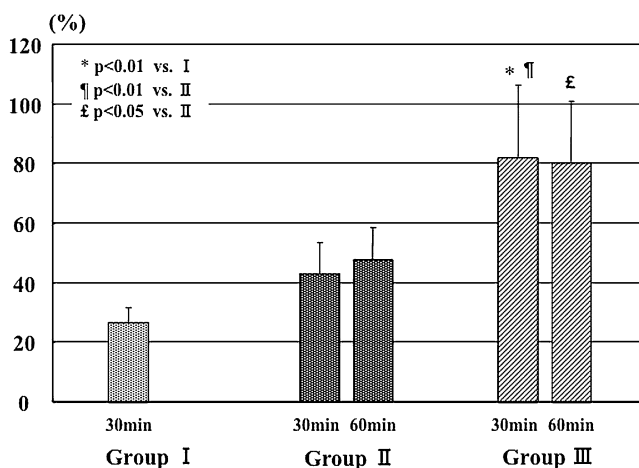
# $P < 0.05$ vs. group II

Fig. 1 Percent recovery of the function curve area from control values [before cardiopulmonary bypass (CPB)]

Figure 1 shows the percent recovery of LV performance assessed by the function curve area. At early reperfusion phase there were no statically significant differences between group I (26.5% ± 5.1%) and group II (42.9% ± 10.8%), signifying incomplete reversal of reperfusion-induced myocardial dysfunction by TWBCP alone. We noted significantly better LV functional recovery in group III (supplemented with olprinone during TWBCP) compared to that in group II (with TWBCP alone) at the late reperfusion phase (80.0% ± 21.1%, $P < 0.05$) as well as at the early reperfusion phase (81.9% ± 24.5%, $P < 0.01$) with no evidence of later deterioration of LV performance in group III.

LV pressure–volume loop

Figure 2A shows the percent recovery of LV contractility (Ees) from pre-CPB control values. Ees in group I was markedly reduced (48.6% ± 5.1%), whereas it was significantly improved in group II with TWBCP at the early and late reperfusion phases (110.2% ± 24.9% and 111.9% ± 19.3%, respectively). Olprinone supplementation in group III showed better recovery of LV contractility during those phases (146.0% ± 33.0% and 147.7% ± 55.3%), although the difference between groups II and III did not reach statistical significance.

Figure 2B shows the percent recovery of LV diastolic performance (tau) from pre-CPB control values. LV diastolic performance was depressed in group I (228.3% ± 48.8%) and restored to the normal range in group II (100.0% ± 12.5%) and group III (92.2% ± 21.9%) ($P < 0.01$ vs. group I). We found no significant difference between groups II and III in terms of supplementation of olprinone during TWBCP.
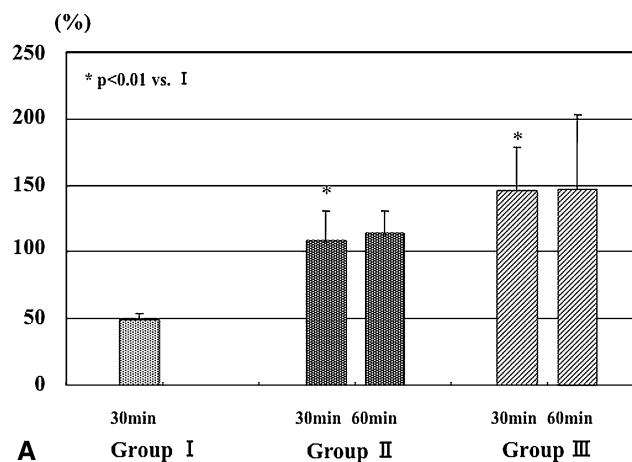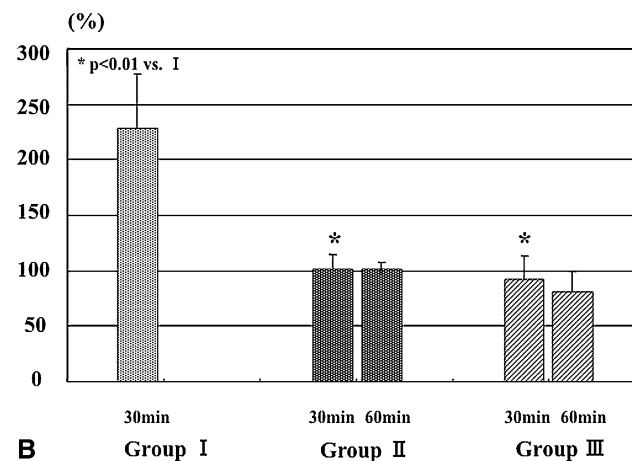
Fig. 2 A Percent recovery of left ventricular (LV) contractility based on the pre-CPB control values. **B** Percent recovery of LV diastolic performance (tau) based on the pre-CPB control values

Arrhythmia

The total quantity of electrical cardioversion for ventricular fibrillation after reperfusion (Fig. 3) was significantly higher in group I (56.0 ± 33.6 J) than in group II (10.0 ± 7.1 J) or group III, where no electrical cardioversion was needed ($P < 0.05$ vs. group II; $P < 0.01$ vs. group III). Group III, supplemented with olprinone during TWBCP. was notable for the absence of arrhythmia, signifying the effect of olprinone on reperfusion-induced arrhythmia.

Biochemical measurements

Figure 4A shows serum concentrations of cardiac troponin-T. Troponin-T concentrations increased after reperfusion and remained above the pre-CPB values throughout the reperfusion phase in groups I and II. In
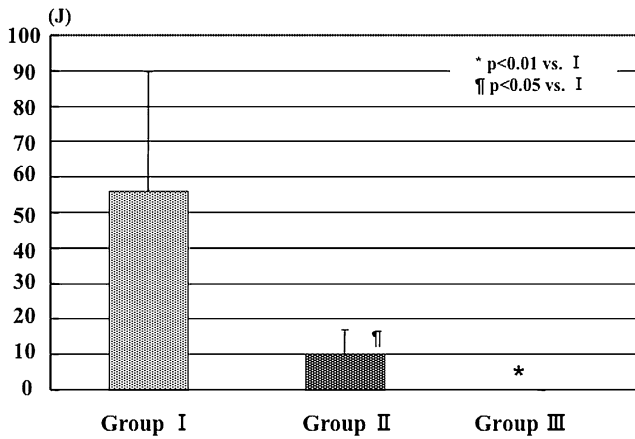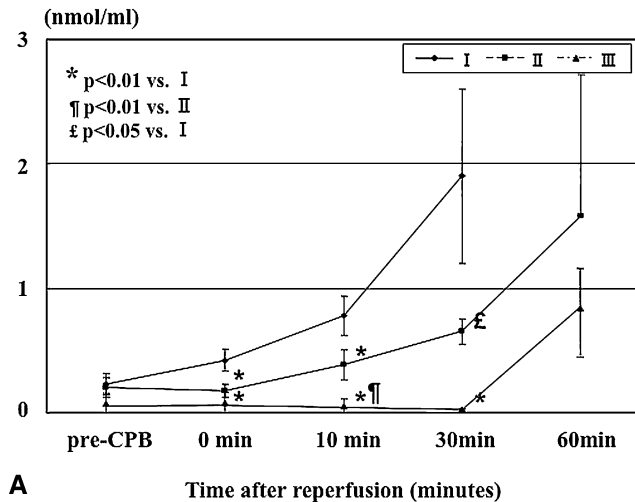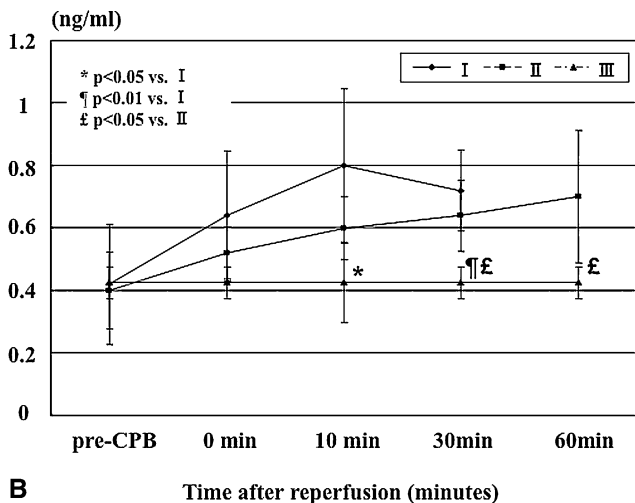
Fig. 3 Total quantity of electrical cardioversion after 90 min of ischemia

Fig. 4 A Serum concentrations of cardiac troponin-T before CPB and after reperfusion. **B** Serum concentrations of lipid peroxide before CPB and after reperfusion

contrast, in group III, supplemented with olprinone, the troponin-T levels were significantly lower than in groups I and II after aortic unclamping ($P < 0.01$). Figure 4B shows serum concentrations of LPO. LPO increased at the onset of reperfusion and remained above pre-CPB values in group I and II. In contrast, LPO remained unchanged after reperfusion in group III. LPO in group III was statistically significantly lower than that in groups I and II.

Olprinone concentrations

Figure 5 shows olprinone concentrations in TWBCP and the time course of serum concentrations of olprinone in group III. The concentration of olprinone was 2126 ± 227 ng/ml in TWBCP, 15 times higher than the usual therapeutic dosage described in the drug information. This was due to the selective administration of high olprinone doses, dropping suddenly after unclamping due to dispersion into the systemic circulation and gradually decreasing thereafter (153.0 ± 37.4, 75.0 ± 29.8, 30.0 ± 6.9, and 14.9 ± 2.6 ng/ml at 0, 10, 30, and 60 min after aortic unclamping, respectively). The myocardial tissue concentration of olprinone in group III 60 min after aortic unclamping at the end of the protocol was 24.4 ± 0.8 ng/g.

Myocardial cAMP content

Cyclic AMP content at the end of reperfusion was significantly lower in group I (0.41 ± 0.12 pmol/mg) than in group II (0.60 ± 0.14 pmol/mg) and group III (0.56 ± 0.14 pmol/mg), whereas no difference was noted between groups II and III at 60 min of reperfusion.

Discussion

We showed that high dose PDE III inhibitor olprinone, selectively administered during TWBCP, provides myocardial protective effects against oxidant-mediated biochemical injury induced by ischemic/reperfusion. It led to prompt and sustained myocardial functional recovery in an open-chest infantile piglet model of prolonged ischemia on CPB. The experimental model in the present study was intended to simulate clinical conditions in which ischemic intervals are unexpectedly prolonged or myocardial protection is inadequate, as TBCP was not helpful in the present study. The modality examined may be useful as rescue or trouble-shooting strategies under these conditions to enhance the effects of TWVCP. Given the concerns about the potential detrimental effects of PDE inhibitors at the early reperfusion phase due to intracellular calcium overload,[15] we first con-

Fig. 5 Olprinone concentrations in terminal blood cardioplegia solution and the time course of serum concentrations of olprinone levels in group III
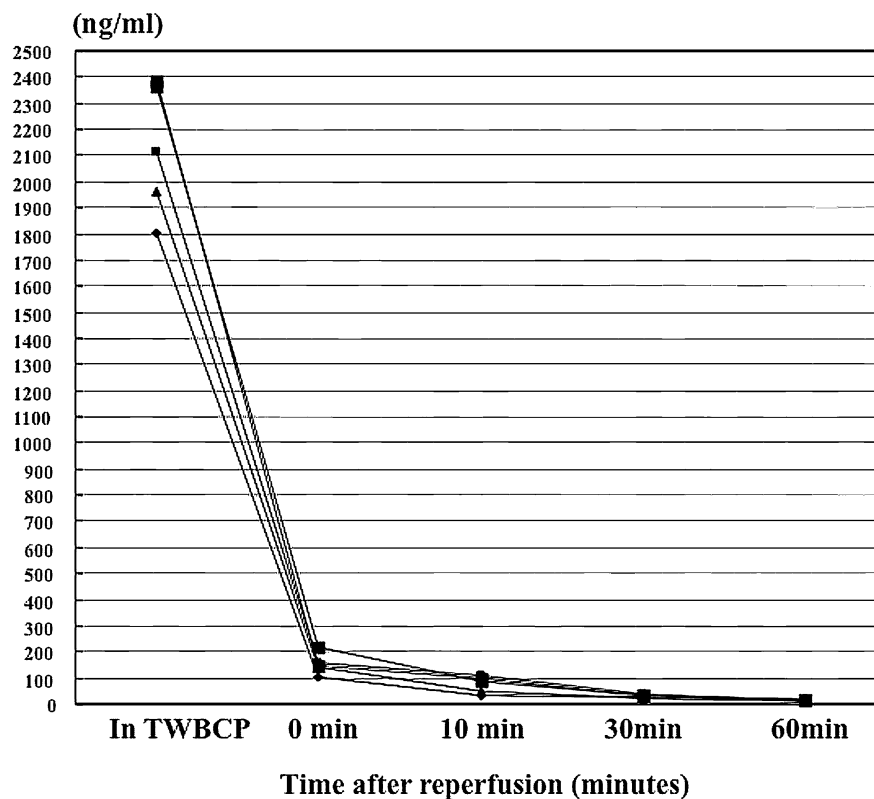
firmed that it is safe and beneficial if incorporated with controlled reperfusion during sustained cardiac arrest with TWBCP.

In hearts, PDE III inhibitors have been shown to be beneficial against ischemic/reperfusion injury if administered before ischemia (pretreatment) in a canine regional ischemic model (coronary occlusion), as reported by Sanada et al.,[13] and during ischemia in isolated rat heart transplantation models, as reported by Besirli et al.[14] Regarding cardioprotection afforded by PDE III inhibitors administered at reperfusion, Chen et al.[7] demonstrated that the use of PDE III inhibitors at the initial reperfusion phase was shown beneficial in an isolated rabbit heart preparation model by its action against leukocyte aggregation. Conversely, Shimada et al.[15] showed in an isolated working rat heart preparation model that continuous infusion of isoproterenol or milrinone before ischemia throughout reperfusion resulted in detrimental effects at high doses, raising potential concerns involving calcium overload during the early reperfusion phase, the most vulnerable time for intracellular calcium overload, which can lead to irreversible myocardial injury.

The elevation of intracellular cAMP levels in cardiomyocytes induced by PDE III inhibitors or others likely results in intracellular calcium overload through the calcium channel and sarcoplasmic reticulum during reperfusion. Upon uncontrolled reperfusion, PDE III inhibitors may phosphorylate the sarcolemmal Ca^{2+} channel protein, increasing calcium permeability and Ca^{2+} entry through the calcium channel, which may lead to release of Ca^{2+} through the sarcoplasmic reticulum (Ca^{2+}-induced Ca^{2+} release), resulting in Ca^{2+} overload when electromechanical activity is restored after unclamping the aorta. Nevertheless, the primary mechanism of Ca overload during early reperfusion is known to be Na^+/H^+ change and Na^+/Ca^{2+} exchange, which are not affected by olprinone, rather than sarcolemmal Ca channel and Ca ATPase in the sarcoplasmic reticulum.

We speculate that at the early stage of reperfusion when the TWBCP solution is infused, the Ca^{2+} channel remains inactivated. PDE III inhibitor administered to the heart arrested with hyperkalemia may facilitate Ca^{2+} entry through the activated Ca^{2+} pumps into the sarcoplasmic reticulum, leading to a reversal of the calcium overload that occurred at reperfusion. This speculation is somewhat supported by data from attenuation of reperfusion injuries and calcium accumulation in the liver due to augmentation of cAMP by administration of the PDE III inhibitor amrinone.[19]

Some discrepancy is noted between Ees and LV function curve recovery between groups II and III. The reason for it was not answered in the present study, but we speculate that PDE III might improve diastolic filling in addition to augmenting systolic contractility (assessed

by Ees), which cannot be assessed by tau, which is the load-dependent index. However, to clarify the mechanism for superior LV function curve recovery in group III, more precise analysis of LV end-diastolic pressure-volume relation (EDPVR) is mandatory.

With respect to the mechanism of functional recovery shown in the present study, the persistence of the direct inotropic and dilatory effects of the high-dose PDE III inhibitor olprinone added to TWBCP should be noted. The initial concentration in TWBCP was set much higher than the usual therapeutic dosage because of our expectation of its antioxidant effect. We were ensuring that the concentration was sufficient to confer inotropic and dilatory effects at the time of restoring the cardiac beat immediately after unclamping.

However, plasma concentrations of olprinone dropped to <200 ng/ml suddenly after unclamping owing to dispersion into the systemic circulation. They gradually declined thereafter, reaching 30.0 and 14.9 ng/ml, which are theoretically assumed to be below the borderline level for inotropic effects of this drug.[20] Therefore, the authors believe that the functional recovery was attributable to effects against reperfusion-induced biochemical injury rather than direct mechanical assistance. This speculation is supported by the fact that functional recovery is associated with reduced biochemical injury, as determined by the measurements of troponin-T and LPO (markers of oxidant-mediated lipid peroxidation).

The data of the concentration-response curves for the effect of olprinone on the force of contraction described in an isolated canine right ventricular trabeculae model by Satoh and Endoh[20] provides the persuasive interpretation that an olprinone concentration of 200 ng/ml (0.66×10^{-6} mol/l) can evoke 110% augmentation of force of contraction and that of 30 ng/ml (10^{-7} mol/l) at functional measurements is far less than threshold of the positive inotropic effect (3×10^{-7} mol/l).[20] Moreover, persistent LV functional recovery was maintained in group III from 30 min to 60 min after reperfusion. Moreover, myocardial olprinone concentrations were similar to plasma concentrations owing to the extremely rapid clearance of olprinone from tissue, including heart muscle, as found by radioactivity studies in rats.[21] Also we do not believe that systemic vasodilatation induced by this drug affected the superior LV functional recovery because there was no difference in the systemic venous resistance between the groups, as shown in Table 2.

One may argue that even though the concentration of this drug dropped below the borderline level for inotropic effects, persistent enhancement of protein kinase A activity and inotropic effects may exist, because the serum and myocardial concentrations of this drug do not represent the actual status of inhibition of PDE III. In response to this issue, we speculate that inhibition of PDE III was no longer present based on the results of the myocardial cAMP level at the end of the observation period (no differences in groups II and III) when the hemodynamic evaluation was undertaken. This observation seems to strengthen the validity of our interpretation of the mechanism of its action, although a definitive conclusion cannot be made because of a lack of serial data of the cAMP content throughout the experimental protocol (during ischemia and reperfusion).

Taking all aforementioned information together, we believe that functional recovery is mainly attributable to effects against reperfusion-induced biochemical injury. We should acknowledge that the initial high dose of PDE III inhibitor during TBCP may somehow confer inotropic effects due to enhancing cAMP and facilitating calcium handling during the early reperfusion phase, contributing to early recovery from stunning.

With respect to the mechanism of olprinone's cardioprotective action, the benefits with respect to reperfusion-induced biochemical injury are linked to various cellular actions. They include reduced production of reactive oxygen species from leukocytes[22,23] and/or direct antioxidant effects (i.e., scavenging oxidants,[5,16] inactivation and inhibition of leukocyte aggregation associated with the no-reflow phenomenon,[7,12] antiplatelet action,[6] and triggering and processing the cellular pathway via protein kinase A and p38 MAPK).[13,24]

Based on study of a canine coronary occlusion model, Sanada et al.[13] reported that pretreatment with transient intravenous exposure to PDE III inhibitors has "ischemic-preconditioning"-like cardioprotective effects via protein kinase A and p38 MAPK. Chen et al.[7] reported that intravenous administration of PDE III inhibitors in isolated rabbit heart models beginning just before the onset of reperfusion has myocardium-protective effects comparable to the effects of inhibiting leukocyte aggregation, suggesting its action against leukocyte infiltration. For an in vitro study using human neutrophils, Mikawa et al.[5] reported that PDE III inhibitors acted by scavenging reactive oxygen species. The effects of TWBCP may be less beneficial after prolonged ischemia, as shown in group II in the present study, associated with inevitable oxidant-mediated injury by this modality alone, as described also by Weisel et al., who had conducted a clinical study.[3] The marked reduction of plasma LPO and troponin T in group III shows that olprinone in TWBCP can reduce oxidant stress at reperfusion due to aforementioned cellular mechanisms and enhance the biochemical benefits of TWBCP, although our studies do not specify the relative contributions of the multiple sources of oxygen-free radicals.

It is noteworthy that no cardioversion was needed at reperfusion in any of the piglets in group III (with olprinone). Lipid peroxidation of the myocardial cell membrane by oxygen-free radicals has been implicated as a potential mechanism of reperfusion-related arrhythmias.[25] The present study, which showed that administration of olprinone reduces serum concentrations of LPO with the benefit of avoiding reperfusion arrhythmia provides further evidence supporting this concept. Rapid recovery to sinus rhythm after aortic unclamping with this modality may be considered enormously advantageous in addition to the excellent hemodynamics obtained in a clinical arena.

Conclusion

Administering the PDE III inhibitor olprinone to supplement TWBCP at the early period of reperfusion in an infantile piglet model of surgical reperfusion attenuated oxidant-mediated myocardial reperfusion injury. The present study indicates that administering this drug directly into an ischemic heart during early reperfusion is cardioprotective. We know of no other study of clinically relevant models involving TWBCP supplements that has addressed the myocardium-protective effects of this PDE III inhibitor. The present study model is clinically relevant because administering this drug is clinically applicable. Further study is needed to determine the optimal dose of delivery to the myocardium.

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