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Abstract

A shortage of donor organs in clinical transplantation prompted us to study whether resuscitated dead hearts could be utilized for successful orthotopic heart transplantation. After 60 min of hypoxic cardiac arrest, one group of canine hearts was resuscitated (Res group, n = 6). The other group was harvested directly (Non-Res group, n = 6). In the Res group, cardiopulmonary bypass was utilized for resuscitation at 37 degrees C and the animals were then core-cooled to 15 degrees C. The hearts then were preserved in University of Wisconsin solution and orthotopically transplanted. Stable prostacyclin analogue (OP2507) and verapamil, a calcium antagonist, were added to the cardioplegia, and substrate-enriched warm blood cardioplegia and a hydroxy radical scavenger (EPC) were administered at the time of reperfusion of the transplanted heart. All animals in each group were successfully weaned from cardiopulmonary bypass with dopamine (5 micrograms/kg/min). Cardiac function without dopamine was better preserved in the Res group than the Non-Res group (Emax: 130.6 +/- 41.5% vs. 47.1 +/- 24.7%; mean +/- SD, as percent of postbrain death values, $P < 0.01$ by unpaired t-test). Cadaver hearts 60 min after anoxic arrest can be successfully re-animated and orthotopically engrafted. In addition, the core-cooling technique is useful. We believe this study serves as the key step in the clinical application of dead hearts to successful cardiac transplantation.

KEYWORDS: heart transplantation, cadaver heart, corecooling, Emax

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Transplantation of the Cadaver Heart Harvested One Hour After Hypoxic Cardiac Arrest Using the Core-Cooling Technique in Dogs

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A shortage of donor organs in clinical transplantation prompted us to study whether resuscitated dead hearts could be utilized for successful orthotopic heart transplantation. After 60 min of hypoxic cardiac arrest, one group of canine hearts was resuscitated (Res group, n = 6). The other group was harvested directly (Non-Res group, n = 6). In the Res group, cardiopulmonary bypass was utilized for resuscitation at 37°C and the animals were then core-cooled to 15°C. The hearts then were preserved in University of Wisconsin solution and orthotopically transplanted. Stable prostacyclin analogue (OP2507) and verapamil, a calcium antagonist, were added to the cardioplegia, and substrate-enriched warm blood cardioplegia and a hydroxy radical scavenger (EPC) were administered at the time of reperfusion of the transplanted heart. All animals in each group were successfully weaned from cardiopulmonary bypass with dopamine (5 μg/kg/min). Cardiac function without dopamine was better preserved in the Res group than the Non-Res group (Emax: 130.6 ± 41.5% *vs.* 47.1 ± 24.7%; mean ± SD, as percent of post-brain death values, $P < 0.01$ by unpaired *t*-test). Cadaver hearts 60 min after anoxic arrest can be successfully re-animated and orthotopically engrafted. In addition, the core-cooling technique is useful. We believe this study serves as the key step in the clinical application of dead hearts to successful cardiac transplantation.

Key words: heart transplantation, cadaver heart, core-cooling, Emax

Cardiac transplantation has been accepted in foreign countries as a successful therapeutic option for patients with intractable chronic heart failure and severe congenital heart disease. However, as the number of heart transplant centers has increased over the last 5 years, the chronic shortage of donor hearts has become critical. The number of heart transplantations has plateaued since approximately 1988 in the United States (1). Successful orthotopic heart transplantation (HTx) has not been performed in Japan since brain death has not yet been accepted as legal death for cultural and religious reasons. If the "dead" heart following cardiac arrest can be used for transplantation, the donor heart pool can be expanded, and cardiac transplantation might be possible in Japan.

Recently, we have reported that donor hearts core-cooled using cardiopulmonary bypass (CPB) following 3 min of normothermic anoxic arrest could be successfully used for heart transplantation. In addition, it was reported that the hydroxy radical scavenger EPC (Senju Pharmaceutical Co., Ltd, Osaka, Japan) protected the cadaver hearts against ischemic damage. In these studies, the resuscitation and core-cooling technique using CPB provided a beneficial effect on post-transplant cardiac function (2, 3).

In the current study, the arrest period was lengthened up to 60 min using myocardial protective agents. The purpose of this study was to evaluate whether cadaver hearts could be re-animated and transplanted after a 1-h arrest, and whether resuscitation by the core-cooling technique is useful.

Materials and Methods

All animals in this study received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal

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Resources and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Two groups, each containing six pairs of dogs weighing 7.0-25.0 kg, were used as donors and recipients in the resuscitation (Res) group (n = 6) and the non-resuscitation (Non-Res) group (n = 6). Donor dogs were anesthetized with ketamine hydrochloride (0.3 mg/kg, i.m.) and sodium pentobarbital (5 mg/kg, i.v.). After endotracheal intubation, the dogs were placed on a volume-cycled ventilator with a tidal volume of 20 ml/kg, a respiratory rate of 12-15 per min, and an FiO_2 of 50 %. Halothane was initiated at 0.5 to 1.0 %. Neurologic examination confirmed that all brain stem reflexes were present at this stage. The right femoral artery and vein were cannulated for systemic arterial pressure monitoring, blood sampling, and infusions. A 5 Fr balloon-tipped thermodilution catheter (Swan-Ganz catheter) was placed into the pulmonary artery through the left femoral vein for measuring cardiac output. The right external jugular vein and carotid artery were dissected for cannulation. A median sternotomy was performed. The azygos vein was ligated and divided. Following pericardiotomy, the vena cavae and ascending aorta were exposed and dissected to prepare for CPB. Heparin was given intravenously (100 U/kg). Two catheters, a conductance (Cordis Europa NV, Roden, The Netherlands) and a high fidelity micro manometer (Model SPC-460; Millar Instruments, Houston, TX USA), were inserted into the left ventricle through the ventricular apex in order to continuously monitor simultaneous left ventricular volume and pressure (4). A 12 Fr vent catheter was placed into the left atrium through the left atrial appendage to monitor the left atrial pressure and for venting during CPB. The baseline functional data that were measured included systemic arterial pressure (AP), pulmonary arterial pressure (PAP), left atrial pressure (LAP), left ventricular end diastolic pressure (LVEDP), cardiac output (CO), left ventricular (LV) max dp/dt, max -dp/dt, and Emax (end-systolic pressure-volume ratio). The cardiac function was quantified by CO, LV max dp/dt, max -dp/dt, and Emax. CO was measured by the thermodilution method with a Swan-Ganz catheter. LVEDP and LV dp/dt were obtained from the continuous LV pressure curve recorded using the high fidelity micro manometer catheter. Emax was measured from the continuous LV pressure-volume relation curve obtained with a volumetric system (Sigma 5; Leycom, Ocgestgeet, The Netherlands) using computer software (Taisho Biomedical Instruments Co., Ltd., Osaka, Japan) during transit acute volume unloading induced by occlusion of both the superior vena cava (SVC) and inferior vena cava (IVC). Calibration for blood conductivity was done just before each measurement.

Under anesthesia, burr hole was drilled into the right frontal region of the skull, the dura mater was incised, and a 24 Fr Foley catheter was introduced into the subdural space. The rapid injection of 10-20 ml of distilled water to inflate the balloon of the catheter produced acute intracranial hypertension and brain herniation. Brain death was confirmed within 20 min (5). The donor dog was kept in a steady state for 60 min after the rapid injection of water, and was maintained with a systolic AP above 80 mmHg by the infusion of fluids or the administration of methoxamine. After confirming brain death by the disappearance of the brain stem reflexes, cardiac function was measured.

In the Res group, the donor dog was then cannulated for resuscitation by CPB. Arterial cannulation was performed through the right carotid artery, and a venous cannula was inserted into the right jugular vein.

The respirator was then disconnected, and the hydroxy radical scavenger EPC (5 mg/kg) (2, 6, 7), the prostacyclin analogue OP2507 (0.02 mg/kg) (8-10) and verapamil (0.25 mg/kg) (11, 12), a calcium antagonist, were given intravenously (13). Cardiac arrest was induced within 10 min because of hypoxia. Cardiac arrest was confirmed as the pulse pressure became zero or when ventricular fibrillation occurred in the donor heart.

In the Res group, 60 min after cardiac arrest, resuscitation by CPB was started at 37 °C. With initiation of CPB, EPC (5 mg/kg) was again administered intravenously. After total assistance of the donor heart for 30 min, the heart was weaned from CPB, the respirator was started temporary, and hemodynamic measurements were taken to confirm the recovery of myocardial function. Then, core-cooling was initiated. After reaching a myocardial temperature of less than 15 °C, the donor hearts were arrested with cold crystalloid cardioplegia (St. Thomas solution 15 ml/kg) containing OP2507 (0.6 mg/l), and verapamil (0.5 mg/l). The vena cavae, the pulmonary vein and artery, and the aorta were divided, and the donor heart was excised. The donor heart was immersed immediately in cold University of Wisconsin (UW) solution containing OP2507 (0.3 mg/l), and preserved at 4 °C.

In the Non-Res group, the hearts were not resuscitat-

ed. Cold crystalloid cardioplegia was induced 60 min after the cardiac arrest, and the donor hearts were harvested without resuscitation and preserved using the same solution as in the Res group.

The anesthesia of the recipient was identical to that of the donor. Following a median sternotomy, the azygos vein was ligated and preliminary dissection was performed. Then, the recipient was heparinized (300 U/kg) and cannulated for CPB using the right carotid artery for arterial cannula and bicaval venous cannulae. CPB was initiated and the recipient was cooled down to 28°C. The recipient's native heart was excised and the donor heart was orthotopically implanted as described by Lower and Shumway (14). The left atrial anastomosis was performed using a running 4-0 polypropylene suture. A 12 Fr vent catheter was placed into the left atrium through the left atrial appendage. Cold crystalloid cardioplegia was given in a retrograde fashion (7.5 ml/kg). The pulmonary artery anastomosis was then performed using a running 6-0 polypropylene suture. Cold crystalloid cardioplegia was given in a retrograde fashion. The aortic anastomosis was performed using a running 5-0 polypropylene suture, rewarming to 37°C was begun. The right atrial anastomosis was performed using a running 4-0 polypropylene suture, and the transplantation was completed. Substrate-enriched warm terminal blood cardioplegia was induced in a retrograde fashion for 5 min and EPC (5 mg/kg) was given again just before unclamping the aorta.

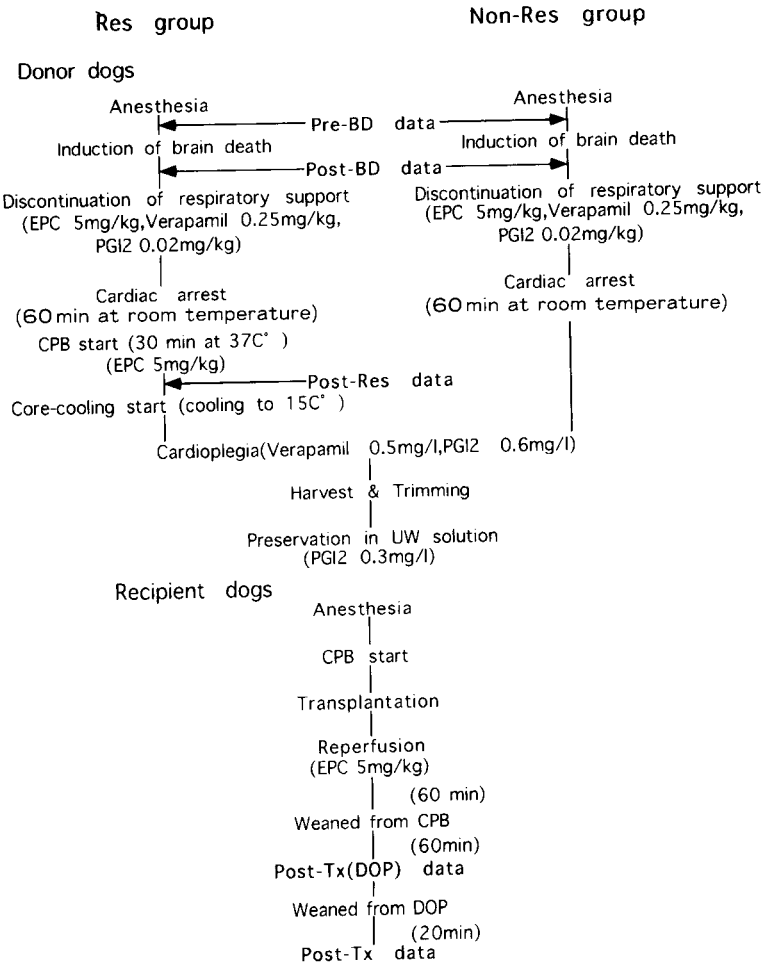

Fig. 1 Experimental protocol. CPB: Cardiopulmonary bypass; DOP: Dopamine; BD: Brain Death; Res: Resuscitation; Tx: Transplantation; Res group: Resuscitation group; Non-Res group: Non-resuscitation group.

Ventricular fibrillation was treated by electric shock and lidocaine (10 mg/kg). Temporary atrial or ventricular pacing was used to maintain the heart rate at more than 80 beats/min, and an inotropic agent (dopamine 5 μg/kg/min) was administered to maintain the systolic AP above 80 mmHg and the LAP below 10 mmHg. The recipient was weaned from CPB after 60 min of non-work beating. The venous cannulae were then removed and a Swan-Ganz catheter was inserted into the pulmonary artery through the right external jugular vein. At 1 h after weaning from CPB, post-transplant cardiac function was measured under two different inotropic states (with and without dopamine 5 μg/kg/min).

The time points of functional data accumulation were pre-induction of brain death (PreBD), post-induction of brain death (PostBD), after resuscitation (PostRes), post-transplantation with dopamine 5 μg/kg/min (PostTx (DOP)), and post-transplantation without inotropic support (PostTx) (Fig. 1).

Aortic blood samples were taken to measure blood gas and creatine phosphokinase MB (CPK-MB) levels. Arterial blood gases were analyzed at 30-min intervals and corrected by the administration of sodium bicarbonate or adjusting the respirator to maintain the pH at 7.4. The CPK-MB level was corrected for hemodilution according to the following formula: corrected value = measured value $\times$ pre-CPB hematocrit/measured hematocrit.

Statistical analysis was performed using Student's t-test to compare unpaired data. Analysis of incidence was performed by the χ^2-test. A probability value of less than 0.05 was considered statistically significant. Values reported are mean $\pm$ one standard deviation (SD).

Results

Cardiac function indices in the donor before and after induction of brain death were not significantly different between the two groups (Figs. 2–5). No significant differences existed between the two groups with respect to the time of hypoxia (from disconnection of the ventilator to cardiac arrest), the time of ischemia (from clamping of the aorta of the donor to reperfusion), or the time of weaning (from reperfusion to cessation of CPB) (Table 1).

After resuscitation, it was impossible to wean three of the six donor hearts (Res group) from CPB. In these cases, after confirming that the heart was beating, core-cooling was initiated. After transplantation, these hearts exhibited stable hemodynamics.

In both groups, the transplanted hearts were weaned from CPB with inotropic support (dopamine 5 μg/kg/min) after non-work beating. Although only one of the hearts in the Non-Res group could be weaned from inotropic support, all of the hearts in the Res group were weaned from the dopamine support while maintaining a systolic AP above 80 mmHg and a LAP below 10 mmHg ($P < 0.05$) (Table 2). The hemodynamics 1 h after weaning CPB was better in the Res group, but the improvements were not significant (Table 2).

In regard to post-transplant cardiac function, the max dp/dt and max-dp/dt were not significantly different

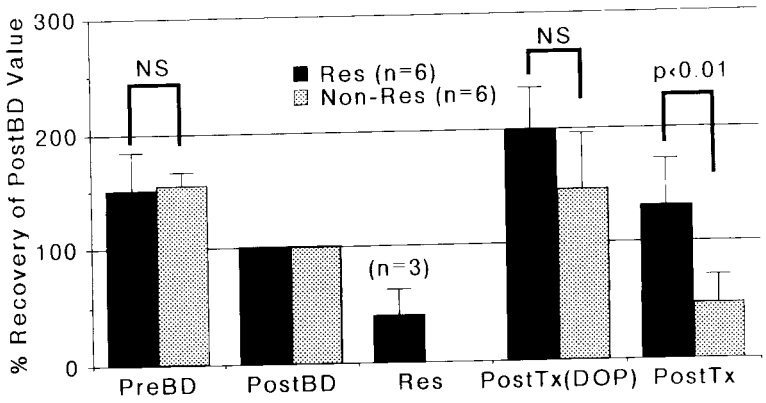

Fig. 2 Comparison between the two groups on percent recovery of Emax (end-systolic pressure-volume ratio).
PreBD: Pre-induction of brain death; PostBD: Post-induction of brain death; PostRes: After resuscitation; PostTx (DOP): Post-transplantation with dopamine (5 μg/kg/min); PostTx: Post transplantation without cathecholamine support.
Values are expressed as percent recovery of post-induction of brain death value and expressed as mean $\pm$ SD.

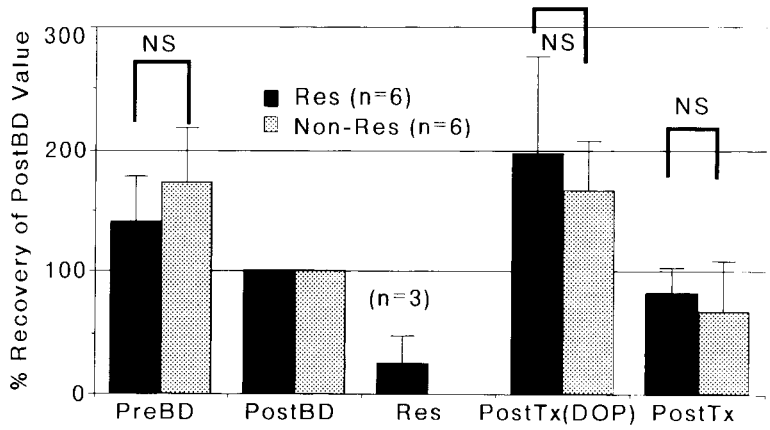

Fig. 3 Comparison between the Res and Non-Res groups on percent recovery of max dp/dt. Abbreviations; See legends to Figs. 1 and 2.

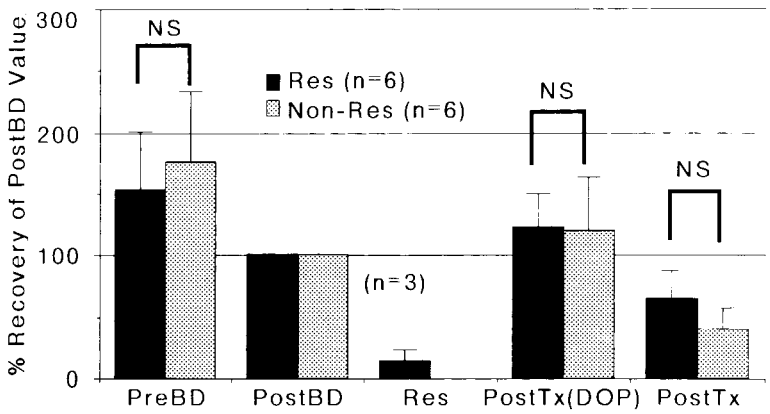

Fig. 4 Comparison between the Res and Non-Res groups on percent recovery of max-dp/dt. Abbreviations; See legends to Figs. 1 and 2.

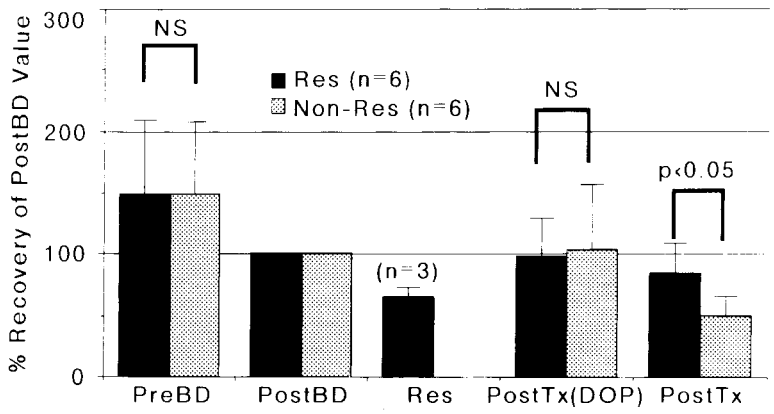

Fig. 5 Comparison between the Res and Non-Res groups on percent recovery of cardiac output. Abbreviations; See legends to Figs. 1 and 2.

Table 1 Procedure variables

	Non-Res (n=6)	Res (n=6)	P
Arrest time (min)	9.7±2.7	10.5±5.3	NS
Ischemic time (min)	292.8±51.8	293.8±31.9	NS
Weaning time (min)	61.7±1.9	63.7±4.0	NS

Res: Resuscitation; Values are expressed as mean ± SD.
NS: not significant.

Table 2 Outcomes and hemodynamics 1 h after CPB

	Non-Res (n=6)	Res (n=6)	P
Weaning from inotropic support	1	6	P=0.019
With DOP			
AP systolic (mmHg)	96.3±22.1	104.3±25.9	NS
AP diastolic (mmHg)	41.7±13.7	54.0±14.3	NS
LAP mean (mmHg)	4.9±1.5	5.2±2.5	NS
Without DOP			
AP systolic (mmHg)	59.6±22.4	78.2±13.0	NS
AP diastolic (mmHg)	32.4±13.5	48.0±10.2	NS
LAP mean (mmHg)	7.2±3.9	6.7±2.9	NS

Res: Resuscitation
CPB: Cardiopulmonary bypass; AP: Arterial pressure; LAP: Left atrial pressure; DOP: Dopamine.
Pressure data were obtained with or without dopamine (5 μg/kg/min). Values are expressed as mean ± SD. NS: not significant.

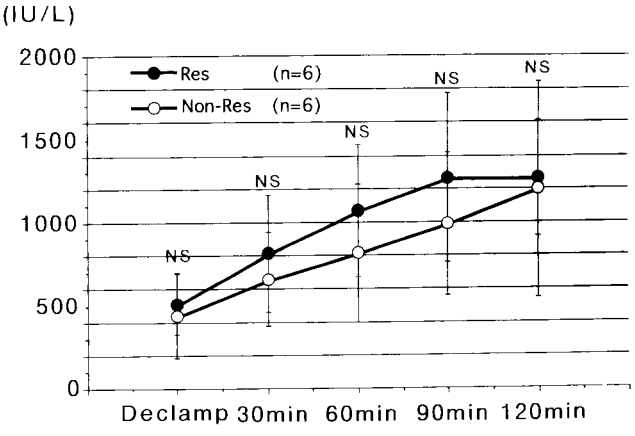

Fig. 6 Comparison of serum CPK-MB concentrations prior to and following reperfusion. CPK-MB: Creatine phosphokinase MB isozyme.

between the two groups regardless of the inotropic state (Figs. 3, 4). However, the Emax and CO in the Res group was significantly better than those in the Non-Res group, even without the inotropic support (Emax: 130.6 ± 41.5 % *vs.* 47.1 ± 24.7 %, $P < 0.01$, CO; 84.1 ± 24.2 % *vs.* 50.2 ± 15.3 %, $P < 0.05$, Figs. 2, 5).

There were no statistically significant differences between the Res and Non-Res groups at any point during the 2 h after unclamping of the aorta (reperfusion) with respect to the CPK-MB level (Fig. 6).

Discussion

According to the registry of the International Society for Heart and Lung Transplantation, the number of heart transplantations in the United States has plateaued since 1988 due to a shortage of donor organs (1, 15). On the other hand, in Japan, clinical transplantation of the heart harvested from the brain-dead donor is not currently accepted. If the cadaver heart can be used for transplantation, the donor pool can be expanded and it may open the way for heart transplantation in Japan.

In 1992, Gundry *et al.* successfully transplanted hearts harvested 30 min after death from exsanguination using cardioplegic solution containing streptokinase in a lamb model (16). Recently, we have demonstrated that donor hearts resuscitated after 3 min of normothermic anoxic arrest and then core-cooled using CPB could be transplanted successfully (2, 3). However, in the clinical situation, 3 min may be long enough to confirm cardiac arrest, but is too short to establish CPB. Additionally, such a short period of normothermic anoxic arrest may not result in significant ischemic damage to the myocardium. Thus, we designed the current study to lengthen the arrest duration up to 60 min. This duration would be long enough to establish CPB and should result in definitive ischemic damage to the myocardium.

In the current study, myocardial protective agents (EPC, prostacyclin analogue, and verapamil with substrate-enriched terminal warm cardioplegia) were used in both groups to attenuate myocardial ischemic damage and reperfusion injury of the cadaver donor hearts.

EPC is a hydroxy radical scavenger (6, 7). A significant amount of evidence has been accumulated to show that free radicals play an important role in the myocardial injury associated with ischemia and reperfusion, and the hydroxy radical is known to be one of the most cytotoxic species of free radicals. Prevention of hydroxy radical-mediated injury may, therefore, improve post-ischemic cardiac function. We have already demonstrated that EPC improves post-transplant cardiac function in this

model (2).

Prostacyclin (OP2507) has anti-platelet and vasodilating actions. Prostacyclin has been reported to have a beneficial effect on myocardial protection under global ischemia with cardioplegia, particularly when used as a component of cardioplegic solution, and also during reperfusion. Its mechanism may relate to the cytoprotective effect (8-10, 13).

Verapamil has been used as an anti-arrhythmic, a vasodilator, and a myocardial protective agent (11, 12). The myocardial protective effect of verapamil is thought to be associated with inhibition of calcium overload, improvement of myocardial blood flow at the time of reperfusion, and attenuation of intracellular acidosis during ischemia (17, 18).

The concept of controlled reperfusion was reported by Buckberg in 1986 (19), who suggested that cardiac muscle damage occurred mainly at reperfusion, not at ischemia. Controlled reperfusion, therefore, can reduce the damage to cardiac muscle after cardiac arrest. He also reported that substrate-enriched terminal warm blood cardioplegia with ventricular decompression using total CPB was very useful to control reperfusion injury.

Using these myocardial protective agents and methods, the cadaver hearts harvested 1h after hypoxic arrest maintained function sufficiently to support recipient circulation, at least in the acute phase.

Moreover, post-transplant cardiac contractile function (Emax) in the Res group was better than that in the Non-Res group. Although other indices (dp/dt, -dp/dt) did not exhibit significant differences, Emax is a load-independent index and is considered to be more suitable for cardiac contractile assessment than max dp/dt and max-dp/dt, which tend to be preload-dependent (20, 21). Thus, we conclude that post-transplant cardiac function in the Res group is well-preserved compared to the Non-Res group. The CPK-MB data shows that there is no detrimental effect in the Res group. The resuscitation and core-cooling technique using CPB provided a beneficial effect on post-transplant cardiac function. This effect was seen in spite of the fact that this technique involves two cycles of global ischemia and reperfusion injury (2, 3).

One important advantage of this model is the capability to assess graft cardiac function after resuscitation in the donor with CPB and separation from bypass. If the post-transplant graft cardiac function is correlated with the post-resuscitation function, it will be very useful for clinical application. Indeed, there is a positive correlation between the post-resuscitation cardiac function and the post-transplant cardiac function (3). In this study, however, this correlation was not observed. Three of six donor hearts in the Res group could not be weaned from CPB at the time of resuscitation. Nevertheless, they could be weaned from CPB after orthotopic transplantation. One possible explanation for this is that donor hearts after 60 min of global ischemia did not fully recover after 30 min of resuscitation. However, with additional protection including EPC and warm blood cardioplegia at the time of reperfusion after transplantation, the donor hearts were able to maintain stable hemodynamics. The impact of the systemic inflammatory effects of the extracorporeal circuit (complement activation, leukocyte sequestration, and free radical-mediated myocardial injury) versus the protective effects of the resuscitation of myocardial function using CPB needs to be elucidated (22).

Although the above-mentioned problems still remain, the results of this study indicate that cadaver hearts 1h after anoxic arrest can be successfully re-animated and orthotopically engrafted, and that the post-transplant cardiac function was better preserved using the core-cooling technique. We believe this experiment serves as the key step in the clinical application of dead hearts for successful heart transplantation.

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