Original Article

Resuscitation and Evaluation of Non-beating Hearts Obtained from Asphyxiated Dogs via Autoperfusing Heart-Lung Circuit

Ken Okamoto, MD,[1] Ryuji Kunitomo, MD,[1] Hirofumi Tagami, MD,[1]
Shuji Moriyama, MD,[1] Ling-Bo Sun, MD,[1] Koji Hirose, MD,[1] Junichi Utoh, MD,[1]
Nobuo Kitamura, MD,[2] and Michio Kawasuji, MD[1]

Background: The shortage of donor hearts has made use of non-beating hearts as cardiac grafts an attractive possibility for heart transplant candidates. The purpose of this study was to evaluate the utility of leukocyte-depleted hot shot cardioplegia for resuscitation of non-beating hearts obtained from asphyxiated dogs via an autoperfusing heart-lung circuit.
Methods: Mongrel dogs were divided into 3 groups according to the warm ischemia time and the method of reperfusion before starting the autoperfusing heart-lung circuit. Group A (n=4) had 60 minutes of warm ischemia and reperfusion without leukocyte-depleted hot shot, Group B (n=5) had 30 minutes of warm ischemia and reperfusion with leukocyte-depleted hot shot, and Group C (n=7) had 60 minutes of warm ischemia and reperfusion with leukocyte-depleted hot shot. We calculated stroke work via the heart-lung circuit to evaluate cardiac function of the resuscitated hearts. The criteria for "recovery" has been reported elsewhere. Myocardial water content of the resuscitated hearts was also measured and analyzed. No inotropic agents were used.
Results: The recovery rates in groups A, B and C were 0%, 80% and 57%, respectively, and the group B rate was significantly higher than the group A rate (p=0.04). Although myocardial water content did not differ between groups B and C, it was significantly lower in recovered hearts than in non-recovered hearts (p=0.04). Significant negative correlation was observed between the maximum stroke work value and myocardial water content in the resuscitated hearts (r=0.668, p=0.03).
Conclusions: The autoperfusing heart-lung circuit is useful for evaluation and maintenance of cardiac function. Our experimental data shows that leukocyte-depleted hot shot plays a great role for resuscitation and recovery of non-beating hearts. (Ann Thorac Cardiovasc Surg 2001; 7: 341–5)

Key words: non-beating heart, autoperfusing heart-lung circuit, warm blood cardioplegia, leukocyte filtration

Introduction

Heart transplantation is the most advanced treatment option for terminal cardiac disease. The number of heart transplants, however, has plateaued since approximately 1989,[1)] and the paucity of donor hearts has become a serious problem. Use of non-beating hearts as cardiac grafts for heart transplantation may be a way to expand donor resources. There are several reports of cadaver heart recovery,[2-12)] but, the limited warm ischemic time,[3,6)] the lack of useful resuscitation techniques, and the lack of proper methods for evaluating resuscitated cardiac function before transplant[6,13,14)] are obstacles to the clinical use of cadaver hearts. With a view to overcoming these problems, we investigated use of leukocyte-depleted hot shot (LDHS) cardioplegia for reanimation of non-beat-

From the [1]First Department of Surgery, Kumamoto University School of Medicine, Kumamoto, and [2]Department of Cardiovascular Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan

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Address reprint requests to Ken Okamoto, MD: First Department of Surgery, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto 860-8556, Japan.

ing canine hearts and evaluated the resuscitated cardiac function via an autoperfusing heart-lung circuit (AHLC).

Materials and Methods

All experiments were approved by the Animal Care Committee of Kumamoto University School of Medicine and performed according to the Guidelines for Animal Experiments of Kumamoto University School of Medicine. All animals received humane care in compliance with the NIH Guide for the Care and Use of Laboratory Animals. No inotropic agent was used in this experiment.

Animal preparation

Adult mongrel dogs of both sexes weighing 11.4 to 17.0 kg (n=16) were anesthetized with ketamine hydrochloride (10 mg/kg, i.m.) and sodium pentobarbital (30 mg/kg, i.v.). The animals were intubated and mechanically ventilated with a tidal volume of 30 ml/kg, a respiratory rate of 15/min., and 50% oxygen. Systemic arterial pressure was monitored in each animal via the right femoral artery. Heparin was injected intravenously at 3 mg/kg body weight after exposure of the heart, and mechanical ventilation was then discontinued. Confirmation of cardiac arrest on the electrocardiogram was decided as the beginning of warm ischemia. The brachiocephalic artery, left subclavian artery, descending aorta, superior and inferior vena cavas, and azygos vein were ligated and divided. The trachea was divided for direct insertion of the tracheal tube, and the heart and lung were carefully excised en block from the chest cavity. An aortic and venous cannula were inserted into the descending aorta and superior vena cava, respectively, to establish the AHLC. Pressure monitoring catheters were inserted into the left subclavian artery and left atrium. After the completion of all cannulation, the heart-lung block was returned to the cadaver chest until the time of warm ischemia.

Blood collection for leukocyte-depleted hot shot and the autoperfusing heart-lung circuit

After heparinization, the right femoral artery was cannulated and approximately 500 to 600 ml whole blood was collected from blood donor dogs (n=16). Animals were anesthetized, intubated, and mechanically ventilated under the same protocol described above. The collected whole blood was diluted with Lactate Ringer's solution to adjust the hematocrit to 25%.

Treatment groups

Animals were divided into three different treatment groups. Group A (n=4) had 60 minutes of warm ischemia and reperfusion without leukocyte-depleted hot shot, Group B (n=5) had 30 minutes of warm ischemia and reperfusion with leukocyte-depleted hot shot, and Group C (n=7) had 60 minutes of warm ischemia and reperfusion with leukocyte-depleted hot shot.

In groups B and C, the diluted blood was passed through a BC-1 filter (Pall Biomedical Products Corporation, Glencoe, NY) to deplete the leukocytes. Only diluted blood without filtration was used in group A.

Leukocyte-depleted hot shot

The leukocyte-depleted blood was oxygenated and mixed one-to-one with GIK (glucose, insulin, and potassium) solution, which consisted of 5% glucose, 20 U/L regular insulin, 40 mEq/L potassium, 0.064% sodium bicarbonate, and 1.2% mannitol, to make a blood cardioplegic solution. Warm blood cardioplegic solution (18 ml/kg) was warmed to 37°C and was injected into the aortic cannula at 60 cm H_2O (LDHS). LDHS returning from the coronary sinus was discarded from the venous cannula.

Autoperfusing heart-lung circuit

For warm ischemia, the aortic and venous cannulas were connected to the AHLC. Transducer outputs from the left atrium and left subclavian artery were displayed on a 6-channel oscillographic monitor Polygraph System (Nihon Kohden, Tokyo, Japan). Cardiac output was measured with an electromagnetic flow probe (Electromagnetic Blood Flowmeter MFV-3200; Nihon Kohden) interposed between the aortic cannula and the circuit. The heart was placed in a thermoregulated water bath maintained at 37°C, and the lungs were ventilated at a tidal volume of 30 ml/kg, a respiratory rate of 15/min, and 50% oxygen.

Stroke work

The AHLC was comprised of two different systems. One maintained coronary circulation without cardiac load (non-loading circuit), and the other calculated stroke work (SW) by changing pre- and afterload (loading circuit). The heart was massaged and defibrillated with a 5-20 J direct current countershock. If the heart began beating and stabilized in the non-loading circuit, the blood was directed to the loading circuit. The desired mean aortic pressure (AP) was obtained by varying the outflow of the loading circuit, and the desired mean left atrial pressure (LAP) was obtained by varying the inflow. SW (gm)

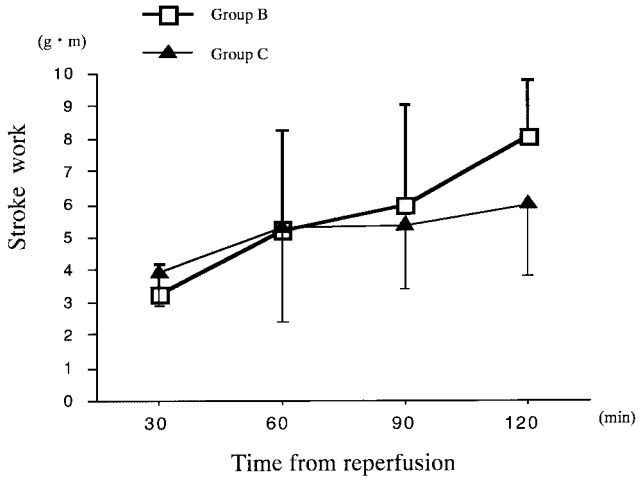

Fig. 1. Change in stroke work (SW) during reperfusion. Groups B and C showed comparable SW patterns (p=0.61). Note the steady rise in SW in these groups.

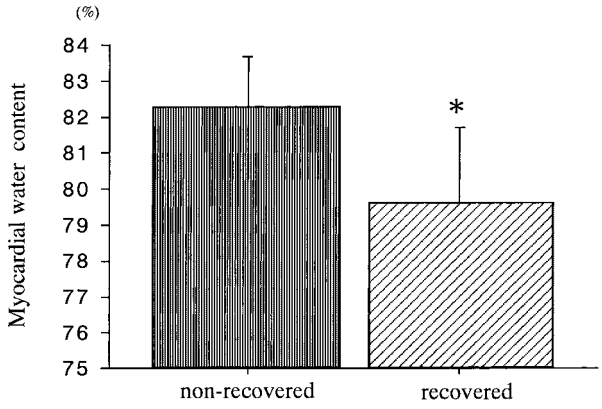

Fig. 2. Myocardial water content in the recovered hearts was significantly lower than that in the non-recovered hearts. recovered: hearts reaching normal function. non-recovered: hearts not reaching normal function. *p<0.05

was calculated as

$$SW=SV\times (AP-LAP)/100$$

where SV denotes stroke volume (ml/beat). SW was calculated at 30, 60, 90, and 120 minutes after reperfusion, and the cardiac function curve was drawn by plotting SW for each LAP. If the curve reached the "normal range" previously reported by Kitamura,[15] the heart was considered recovered.

Myocardial water content

At the end of each experiment, a specimen of left ventricular wall was taken and weighed as wet weight. The specimen was dried for 7 days at 60°C. Myocardial water content (MWC) was calculated according to the following equation:

$$MWC = (\text{wet weight} - \text{dry weight}) / \text{wet weight} \times 100.$$

Statistical analysis

The between-group recovery rate was analyzed by χ^2 test. The time course of the change in SW was analyzed by two-way repeated measures ANOVA (analysis of variance) between groups B and C. MWC was compared by two-tailed unpaired t test between groups B and C and between recovered hearts and non-recovered hearts. Simple regression analysis was performed to determine any association between maximum SW and MWC for all resuscitated hearts. All values are shown as mean ± standard deviation (SD). The StatView 4.5 program (Abacus Concepts, Inc., Berkeley, CA) was used for statistical analysis. Differences were considered significant at p values less than 0.05.

Results

Cardiac arrest ranged from 10 to 29 minutes (mean, 17.8 minutes).

Recovery rate

All 4 hearts in group A (100%), 0 of 5 in group B (0%), and 2 of 7 in group C (29%) developed "stone heart" after reperfusion and could not be evaluated. All 5 hearts in group B (100%) and 5 of 7 in group C (71%) were resuscitated with the AHLC, and 4 of 5 in group B (80%) and 4 of 7 in group C (57%) reached the "normal range" of function. The only significant difference in recovery rate was that between groups B and A (p=0.04).

Stroke work

The time course of changes in SW in groups B and C is shown in Fig. 1. The mean SW values did not differ between these groups (p=0.61) during the observation period.

Myocardial water content

MWC in groups B and C was 80.8±2.2% and 80.3±2.5%, respectively, and the difference was not significant. MWC in the recovered hearts, however, was 79.6±2.1% and significantly lower than that in the non-recovered hearts (82.3±1.4%, p=0.04; Fig. 2). There was significant negative correlation between the maximum SW value and MWC in the resuscitated hearts (r=0.668, p=0.03; Fig. 3).

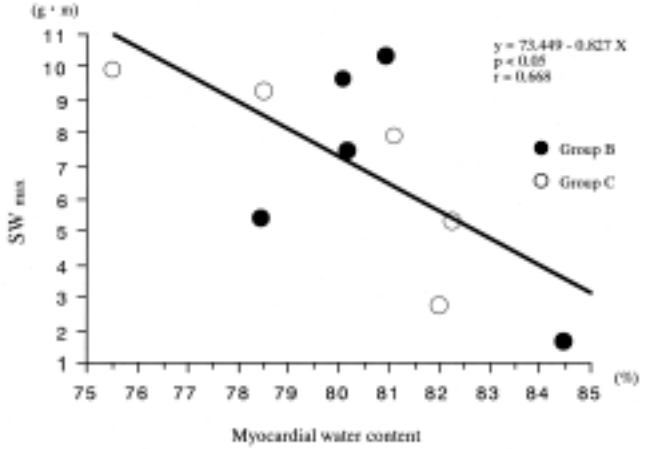

Fig. 3. A close correlation is observed between maximum stroke work (SW_{max}) and myocardial water content in the resuscitated hearts.

Discussion

Since arrested infant and pediatric hearts have been successfully transplanted after cardiopulmonary resuscitation,[16-18] the use of non-beating hearts as cardiac grafts for heart transplantation is anticipated. If successful, problems such as the paucity of donor hearts and the controversial definition of death (that is, "brain" vs "cardiac") can be solved. The next step is establishment of the resuscitation technique and a method for evaluating the function of resuscitated non-beating hearts before transplantation. Several methods for monitoring the viability of the heart have been reported, such as by myocardial electrical impedance,[19] intramyocardial pH,[20] and ^{31}P-NMR spectroscopy.[21] Though these methods may be close to accurately assessing cardiac function,[22] we would hesitate to perform a transplant unless the function of the resuscitated heart is verified. Thus, we experimented with the AHLC. Reliability of the circuit has already been reported,[15,23] and SW, the most important factor for evaluation of cardiac function, can be calculated repeatedly, non-invasively, and accurately. The circuit is also very useful in maintaining cardiac function. In our study, once the heart was resuscitated, SW was maintained for over 2 hours without exchanging the circuit blood (Fig. 1).

In our previously reported exsanguination model experiments, 100% of arrested hearts recovered after 60 minutes of warm ischemia with LDHS, and 25% recovered without LDHS.[23] These results prompted us to try an anoxically arrested model because such hearts are considered the poorest among the available non-beating hearts. Eighty percent of our anoxically arrested hearts reached normal function after 30 minutes of warm ischemia, and 57% of those even after 60 minutes with LDHS. Our results are in accord with those of Gundry et al., who successfully transplanted baboon hearts 15 to 31 minutes after cardiac arrest using the same anoxic model.[8] They also used leukocyte-depleted terminal blood cardioplegic solution before reperfusing the donor hearts. Terminal warm blood cardioplegia or "hot shot" cardioplegia[24] may prove very useful for such a model because the energy stores of the heart are consumed and depleted during anoxic arrest.

In addition to the effect of the hot shot energy charge, leukocyte depletion may have a beneficial effect in these hearts. During reperfusion, leukocytes bind to the coronary vascular endothelium exposed to hypoxia or ischemia,[25] and they release deleterious substances, causing reperfusion injury.[26] Although only LDHS was used in our study, unlike that of Gundry et al.[8] in which other agents such as methylprednisolone, nifedipine and prostaglandin E1 were used, high recovery rates were obtained. LDHS may be the key treatment for resuscitation and recovery of non-beating hearts.

MWC was significantly low in our recovered hearts and was also negatively correlated with the maximum SW value. Since MWC is related to histologic condition and left ventricular filling volume,[27] myocardial edema may be an important factor in reduced SW.

In conclusion, AHLC was useful for evaluation of resuscitated cardiac function. LDHS plays a great role for resuscitation and recovery of non-beating hearts. If these methods can be applied clinically, resuscitation, strict evaluation, and maintenance of non-beating hearts for transplant may also be feasible.

References

1. Hosenpud JD, Bennett LE, Keck BM, Fiol B, Novick RJ. The registry report of the International Society for Heart and Lung Transplantation: fifteenth official report-1998. *J Heart Lung Transplant* 1998; **17**: 656–68.
2. Wuerflein RD, Shumway NE. Resuscitation and function of the cadaver heart. *Circulation* 1967; **35** (4 suppl): I 92–5.
3. Copeland J, Kosek JC, Hurley EJ. Early functional and ultrastructural recovery of canine cadaver hearts. *Circulation* 1968; **37** (4 suppl): II 188–200.
4. Tam W, Robicsek F, Daugherty HK. The autoperfusing heart-lung preparation: a vehicle for the preservation of the resuscitated cadaver heart. *J Thorac Cardiovasc Surg* 1969; **58**: 879–85.
5. Rikkers LF, Chartrand C, Angell WW. Donor shock

and its influence on canine cadaver heart transplants. *Chest* 1971; **59**: 428–32.
6. Cooper DK. A simple method of resuscitation and short-term preservation of the canine cadaver heart. *J Thorac Cardiovasc Surg* 1975; **70**: 896–908.
7. Gundry SR, Alonso de Begona J, Kawauchi M, Liu H. Transplantation and reanimation of hearts removed from donors 30 minutes after warm, asystolic death. *Arch Surg* 1993; **128**: 989–93.
8. Gundry SR, Fukushima N, Eke CC, Hill AC, Zuppan C, Bailey LL. Successful survival of primates receiving transplantation with "dead," nonbeating donor hearts. *J Thorac Cardiovasc Surg* 1995; **109**: 1097–102.
9. Cope JT, Mauney MC, Banks D, et al. Intravenous phenylephrine preconditioning of cardiac grafts from non-heart-beating donors. *Ann Thorac Surg* 1997; **63**: 1664–8.
10. Takagaki M, Hisamochi K, Morimoto T, Bando K, Sano S, Shimizu N. Successful transplantation of cadaver hearts harvested one hour after hypoxic cardiac arrest. *J Heart Lung Transplant* 1996; **15**: 527–31.
11. Cope JT, Mauney MC, Banks D, Binns OA, De Lima NF, Buchanan SA. Controlled reperfusion of cardiac grafts from non-heart-beating donors. *Ann Thorac Surg* 1996; **62**: 1418–23.
12. Fukushima N, Shirakura R, Chang J, et al. Successful multiorgan transplants from non-heart-beating donors using percutaneous cardiopulmonary support. *Transplant Proc* 1998; **30**: 3783–4.
13. Arikawa K. Cadaver heart preservation and transplantation: an experimental study. *J Jpn Assn Thorac Surg* 1980; **28**: 1643–53.
14. Hamada Y. The important factor in preservation of cadaver heart: an experimental study. *J Jpn Assn Thorac Surg* 1980; **28**: 1654–67.
15. Kitamura N. Experimental studies on heart preservation and viability test with a heart-lung preparation--parallel circuits for heart preservation and viability testing. *J Jpn Assn Thorac Surg* 1976; **24**: 36–46.
16. Kawauchi M, Gundry SR, Alonso de Begona J, Razzouk AJ, Bailey LL. Utilization of pediatric donors salvaged by cardiopulmonary resuscitation. *J Heart Lung Transplant* 1993; **12**: 185–8.
17. Boucek MM, Mathis CM, McCormack J, Gundry SR, Bailey LL. Sudden infant death syndrome (SIDS) and heart transplant donors: evidence against an intrinsic cardiac abnormality [abstract]. *Circulation* 1990; **82** (suppl): III-352.
18. Alonso de Begona J, Gundry SR, Razzouk AJ, Boucek MM, Kawauchi MK, Bailey LL. Transplantation of hearts after arrest and resuscitation: early and long-term results. *J Thorac Cardiovasc Surg* 1993; **106**: 1196–201.
19. Ishikawa M, Hirose H, Sasaki E, et al. Noninvasive detection of the limitation of simple cold storage of the heart by myocardial electrical impedance. *J Jpn Assn Thorac Surg* 1995; **43**: 1579–86.
20. Saitoh K, Makino S, Kanamori Y, Shikano K, Yada I, Kusagawa M. Experimental studies on evaluation of myocardial viability using continuous intramyocardial pH measurements during hypothermic heart preservation. *J Jpn Assn Thorac Surg* 1988; **36**: 1307–10.
21. Pohost GM. Is ^{31}P-NMR spectroscopic imaging a viable approach to assess myocardial viability? *Circulation* 1995; **92**: 9–10.
22. Yamanaka S, Shida T, Wakita N, et al. Assessment of the viability of a preserved heart-birefringence test with a polarizing microscope. *Nippon Geka Gakkai Zasshi* 1987; **88**: 1105–13.
23. Okamoto K. Resuscitation and salvage of cardiac grafts from non-heart-beating donors using autoperfusing heart-lung circuit. *Ishoku* 2000; **35**: 168–75.
24. Lazar HL, Buckberg GD, Manganaro AM, Becker H. Myocardial energy replenishment and reversal of ischemic damage by substrate enhancement of secondary blood cardioplegia with amino acids during reperfusion. *J Thorac Cardiovasc Surg* 1980; **80**: 350–9.
25. Lucchesi BR. Modulation of leukocyte-mediated myocardial reperfusion injury [review]. *Annu Rev Physiol* 1990; **52**: 561–76.
26. Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am J Pathol* 1982; **107**: 395–418.
27. Carter YM, Jia CX, Soto PF, et al. Diastolic properties, myocardial water content, and histologic condition of the rat left ventricle: effect of varied osmolarity of a coronary perfusate. *J Heart Lung Transplant* 1998; **17**: 140–9.