Transplant Int (1989) 2: 78-83

TRANSPLANT
International


# The effect of controlled reperfusion in porcine hearts submitted to three hours of cold global ischemia

A. Haverich, L. Dammenhayn, M. Jurmann, J. Laas, and R. Hoppe

Medizinische Hochschule Hannover, Klinik für Thorax-Herz und Gefäß-Chirurgie, Zentrum Chirurgie, Konstanty-Gutschow-Strasse 8, D-3000 Hannover, Federal Republic of Germany

**Abstract.** At present, many investigations of myocardial function following ischemic insults concentrate on the modalities of reperfusion rather than on the mode of preservation. In this study, we tried to define the effect of reperfusion using warm blood cardioplegia (WBC) after medium-term (3 h) cold global ischemia, as required in cardiac transplantation. Twenty-one porcine hearts were harvested after preservation with cold cardioplegia (St. Thomas Hospital solution) and topical cooling. Normothermic reperfusion with blood was initiated after 3 h of ischemia utilizing a special extracorporeal pump circuit. Twelve hearts served as controls (group A), while substrate-enriched WBC was applied during the initial 20 min of reperfusion in nine hearts (group B). Hearts in both groups were then studied for myocardial function and metabolism under both working and nonworking conditions for a maximum of 180 min. In the nonworking mode, left ventricular dp/dt was significantly higher in group B than in group A at 15 min (2201 ± 785 mm Hg/sec vs 1515 ± 732 mm Hg/sec) and at 180 min (1730 ± 471 mm Hg/sec vs 836 ± 147 mm Hg/sec; $P < 0.05$). After 3 h, lactate production was significantly higher in group A (371 ± 45 mg/dl) than in group B (108 ± 44 mg/dl; $P < 0.05$). Creatine kinase release into the coronary sinus was also significantly elevated in group A at 15 min (2807 ± 1478 IU/l vs 1148 ± 1272 IU/l; $P < 0.05$). Similarly, the hemodynamic data obtained under working conditions in group B were superior to those in group A. We conclude that following 3 h of cold global ischemia, reperfusion with WBC improves myocardial function and metabolism. Cautious application in clinical heart transplantation is recommended.

**Key words:** Heart preservation - Heart transplantation, experimental - Reperfusion injury - Warm blood cardioplegia.

A strong correlation between survival of cardiac transplant recipients and duration of graft ischemia has been established by the large number of cases available in the registry of the International Society for Heart Transplantation [12]. These cases reveal a threefold increase in early mortality after transplantation of hearts submitted to 3-4 h of ischemia (15.7%) as compared to those with less than 1 h of storage (4.9%). Therefore, myocardial preservation of normal hearts, as required in clinical transplantation including distant organ procurement, still needs to be improved.

Myocardial preservation of a donor heart, from the moment of retrieval until the moment transplantation has been completed, involves three major steps: initial preservation with cardioplegic arrest, cold storage, and reperfusion. In the past, the vast majority of both experimental and clinical investigations of myocardial preservation focused only on the first step - cardioplegic solutions - for conventional cardiac surgical procedures as well as for transplantation. Only recently have efforts been made to define optimal modalities of reperfusion in a previously ischemic myocardium. One approach is the method of warm blood cardioplegia (WBC) during the initial phase of reperfusion, as described by Buckberg et al. [5]. In this series of experiments, the importance of controlling reperfusion modalities with respect to composition of the reperfusate (pH, ionic composition, osmolarity, substrates), reperfusion pressure, and functional state of the myocardium during reperfusion was described. In the pres-

*Offprint requests to:* A. Haverich

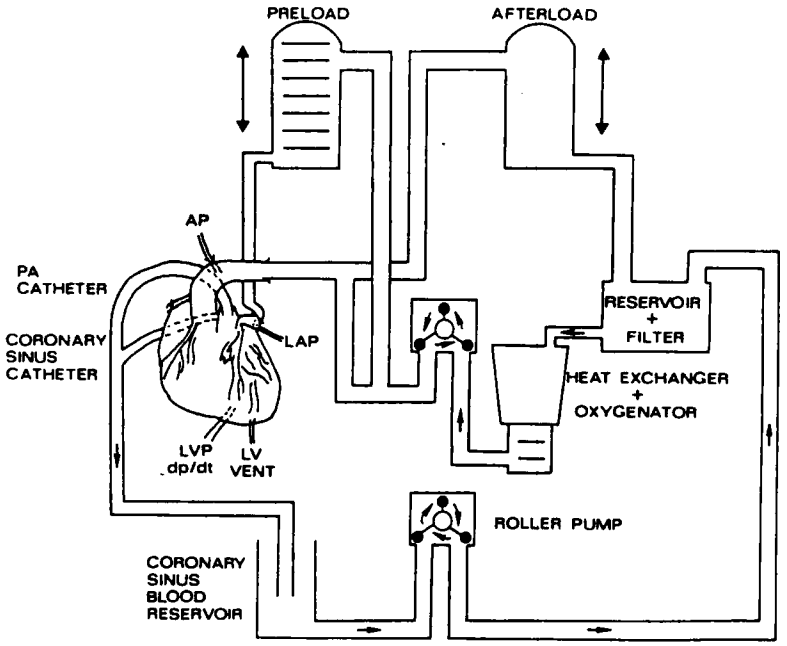


**Fig. 1.** Isolated porcine heart model and extracorporeal perfusion circuit. The circuit consists of a venous line (coronary blood, blood expelled during orthograde perfusion), a roller pump, an oxygenator, and a tubing system. The heart can be perfused via the aortic cannula (retrograde) or via the left atrium (preload reservoir, orthograde perfusion). The coronary sinus blood is sampled in the right atrium and pulmonary artery (*PA*). Both vent-catheter and a pressure-tip catheter, for estimation of left ventricular pressure (*LVP*) and *dp/dt*, are inserted in the left ventricle (*LV*). During orthograde perfusion, the left ventricle ejects blood via the aortic cannula into the afterload reservoir, the height of which [aortic pressure (*AP*)] is variable

ent experimental study, the beneficial effects of WBC, as described for salvage of regional myocardial ischemia, were studied after prolonged cold global ischemia, as required in the clinical setting of heart transplantation.

## Materials and methods

Twenty-one hearts from healthy pigs (25-35 kg body weight) were harvested via a median sternotomy. Both caval veins and the pericardial reflection were transected and cold (4°C) cardioplegic solution was administered into the aortic root at a volume of 20 cc/kg with an aortic root pressure of 70 mm Hg after cross-clamping of the ascending aorta. Simultaneously, cold (4°C) Ringer's solution was poured into the pericardium for surface cooling. At the end of perfusion, the ascending aorta, pulmonary arteries, and pulmonary veins were transected. Hearts were then placed in plastic bags filled with cold (4°-6°C) Ringer's solution for subsequent storage (3 h).

After 2.5 h of hypothermic ischemia, the hearts were prepared by insertion of catheters into the coronary sinus, left atrium, pulmonary artery, and aorta. In addition, pressure monitoring lines were installed in the aortic root, left atrium, and left ventricular cavity using Millar-Tip catheters. The hearts were then mounted on a specially designed perfusion circuit (Fig. 1), which was primed with 1.5 l of blood from the same donor and additional Ringer's solution (1.5-2 l). This system allows for perfusion of the heart in the working or nonworking mode while it is beating spontaneously. During retrograde perfusion (nonworking mode), the blood was drained from the coronary sinus (CS) and collected in a reservoir for quantification and blood sampling. Following oxygenation (BOS 10 Bentley, Irvine, Calif), the blood was pumped back to the heart through the aortic cannula (mean pressure 75 mm Hg). Under conditions of orthograde perfusion (working mode), the heart was filled via the left atrium.

**Table 1.** Composition of warm blood cardioplegia (WBC). Hct, Hematocrit; KCl, Potassium chloride; Bic, Bicarbonate; CPD, Citrate phosphate dextrose

| | |
|---|---|
| Crystalloid: blood 1:4 | |
| Final concentration: | |
| Hct | 20%-30% |
| KCl | 16 m*M*/l |
| pH (Bic) | 7.5-7.6 |
| Glucose | 400 mg% |
| Aspartate | 13 m*M*/l |
| Glutamate | 13 m*M*/l |
| CPD | 10 cc/l |
| Osmolarity | 400 mosmol/l |

After an initial 30-min period of retrograde perfusion, the system was switched to the working state every half hour for about 5 min. Hemodynamic parameters were assessed at 30-min intervals, both in the working and nonworking modes. These parameters included aortic pressure (AP), left atrial pressure (LAP), left ventricular developed pressure (LVDP), and the first derivative of LV pressure (dp/dt). For measurements in the nonworking mode, the left ventricle was filled to an end-diastolic pressure of 5 mm Hg. During orthograde perfusion, mean LAP was increased to 20 mm Hg and the hearts were ejecting against a variable afterload (height of afterload reservoir). Measurements were taken after steady-state conditions were obtained. Experiments were terminated if a left ventricular systolic pressure of 75 mm Hg could not be reached for either perfusion mode. Blood samples from the CS were obtained at regular intervals and analyzed for creatine kinase and lactate using routine oxymetric tests (standard test kits from Bœhringer, Mannheim, FRG). Values achieved were corrected for hematocrit.

Two groups of hearts were studied. The 12 hearts that made up group A served as controls. These hearts had been reperfused with blood from the circuit for the entire study period of 3 h. The 9 hearts in group B were reperfused with WBC for the first 20 min at a pressure of 60 mm Hg. This mode of reperfusion, including the concentration of the ingredients, was adapted entirely from that employed by Buckberg et al. [5]. Thereafter, coronary perfusion via the extracorporeal circuit was established. The final concentration of ions and substrates in the WBC [5] is listed in Table 1.

### *Humane animal care*

All animals received humane care in compliance with the *Principles of laboratory animal care*, formulated by the National Society for Medical Research, and with the *Guide for the care and use of laboratory animals*, prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH) publication 80-23, revised 1978.

### *Statistical analysis*

Results were expressed as mean ± the standard error of the mean (SEM). Data analysis was done using Student's *t*-test for unpaired data with $P<0.05$ considered significant (two groups). A repeated measure analysis of variance to investigate the time released changes between groups could not be used since several hearts in both groups were lost at various intervals during the study period and complete sets of data are needed for this statistical procedure.

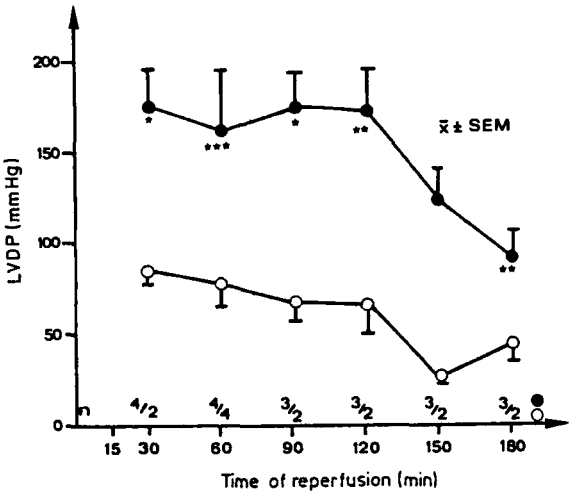


**Fig. 2.** Left ventricular developed pressure (*LVDP*) in the working mode (orthograde perfusion) with time of reperfusion (min). *n*, Number of hearts studied in both groups at respective intervals. * $P<0.01$; ** $P<0.025$; *** $P<0.05$; ○, Controls; ●, WBC

## Results

### *Duration of experiments*

Of the 12 hearts in the control group (group A), 7 could be assessed for left ventricular function in the nonworking mode at 120 min of reperfusion and 3 at 180 min. Four of the 9 hearts in group B were still able to beat during retrograde perfusion at 120 min and 3 could still do so at 180 min. Only 2 of the 12 group A hearts and 3 of the 9 group B hearts could also be followed in the working mode for more than 120 min (Fig. 2). Thus, there was a considerable loss of data in both groups due to myocardial failure, with slightly higher survival in group B.

### *Left ventricular function*

In the nonworking mode, LVDP was consistently higher in group B than in controls (Table 2). A slight decrease in LVDP was seen in both groups with time of reperfusion. In the working mode, this difference between groups increased in favor of group B (Fig. 2). During the first 2 h of reperfusion, LVDP in group B remained above 120 mm Hg, while that in group A hearts remained below 100 mm Hg. Beyond 2 h of reperfusion, a steady decline in LVDP was observed in both groups. Left ventricular dp/dt during retrograde perfusion was also higher in group B than in group A (Table 2). Again, this disparity increased when data obtained during orthograde perfusion was considered. Here, dp/dt values of more than 2000 mm Hg/sec were assessed consistently in the WBC group while none of the control hearts reached 1000 mm Hg/sec after 60 min of reperfusion (Fig. 3).

### *Coronary blood flow*

Coronary blood flow (CBF), as measured during retrograde perfusion, showed a steady increase in group B from 60 ± 10.0 ml/min per 100 g at 15 min to 120 ± 22.2 ml/min per 100 g at 150 min of reperfusion. In the control group, by contrast, a stepwise decrease was noted towards the end of the experiments (77 ± 11.5 ml/min per 100 g). Due to the high variance of data in both groups, no significant differences could be established for individual data points (Fig. 4).

**Table 2.** Systolic left ventricular function during retrograde perfusion (mean ± 1 SEM). LVDP, left ventricular developed pressure

| Time of reperfusion (min) | LVDP (mm Hg) | | | | | Dp/dt (mm Hg/s) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group A | *n* | Group B | *n* | *P* | Group A | *n* | Group B | *n* | *P* |
| 30 | 103 ± 7 | 10 | 152 ± 4 | 8 | <0.0005 | 1849 ± 228 | 10 | 2135 ± 345 | 8 | NS |
| 60 | 103 ± 9 | 7 | 133 ± 15 | 6 | NS | 1675 ± 242 | 6 | 2206 ± 710 | 6 | NS |
| 90 | 94 ± 5 | 6 | 142 ± 9 | 4 | <0.0005 | 1367 ± 123 | 6 | 1997 ± 377 | 3 | NS |
| 120 | 84 ± 8 | 7 | 147 ± 4 | 4 | <0.0005 | 1133 ± 105 | 7 | 2007 ± 303 | 3 | NS |
| 180 | 53 ± 5 | 5 | 120 ± 2 | 4 | <0.0005 | 836 ± 65 | 5 | 1730 ± 336 | 4 | <0.0125 |

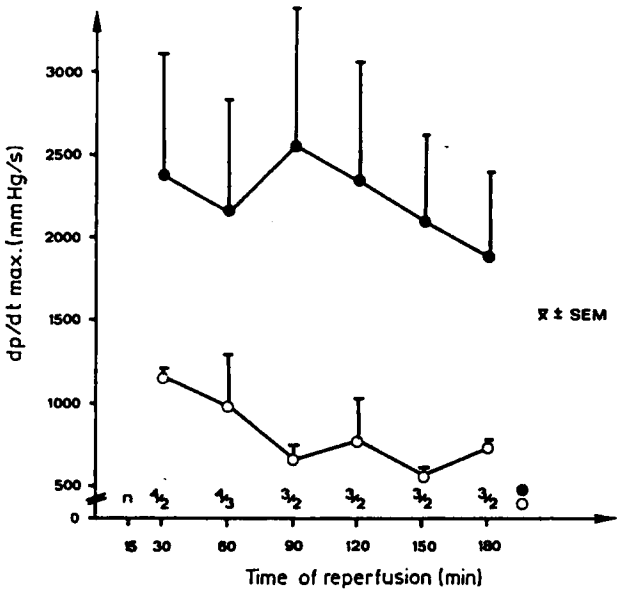


Fig. 3. Maximum left ventricle dp/dt (mm Hg/sec) in the working mode with time of reperfusion (min). *n*, Number of hearts studied in both groups at respective intervals. Symbols as for Fig. 2

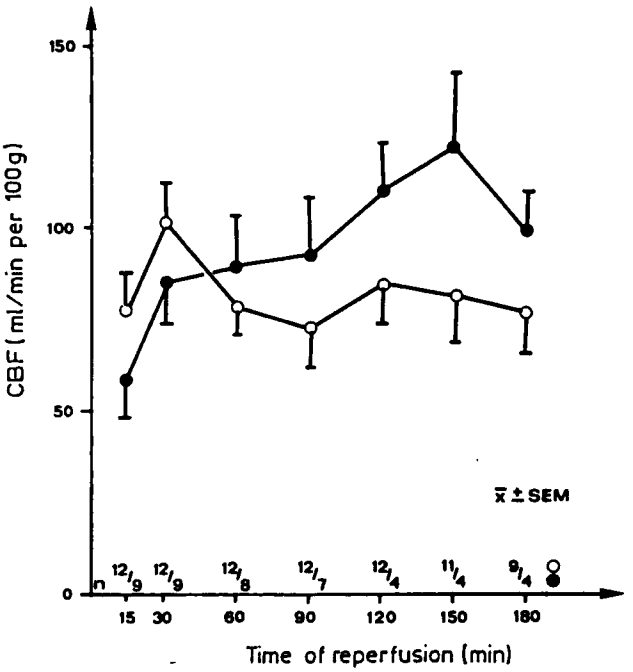


Fig. 4. Coronary blood flow (CBF; ml/min per 100 g) in both groups with time of reperfusion (min). *n*, Number of hearts studied at respective intervals. Symbols as for Fig. 2

### *Myocardial oxygen consumption*

After 15 min, myocardial oxygen consumption ($MVO_2$) was significantly higher in group A than in group B. At 60 min, comparable data were observed in both groups (Table 3). Thereafter, a significant decline in $MVO_2$ could be observed in control hearts. Hearts in group B increased their $MVO_2$ to 179 ± 21.2 ml/min per 100 g at 120 min, significantly higher than that of group A (104 ± 14.4 ml/min per 100 g; $P < 0.025$) at this point.

### *Lactate production*

In group B, lactate concentration in the CS blood showed a very constant level (about 100 mg/dl) throughout the experiment. Conversely, the data in group A demonstrated a steady increase with time of reperfusion and at a significantly higher level at all intervals measured (Table 3). After 180 min, lactate concentration was 371 ± 44.6 mg/dl in group A and 108 ± 43.8 mg/dl in group B ($P < 0.005$).

### *Creatine kinase release*

Throughout the experiment, creatine kinase levels were consistently higher in the control group. At 15 min, values were 2307 ± 1478 IU/l in group A and 1148 ± 1272 IU/l in group B. Thereafter, all hearts showed a steady increase in this enzyme, one which was significantly steeper in group B (Table 3).

## Discussion

We and most other cardiac transplant surgeons consider cold ischemia times of 3–4 h for donor hearts to be safe for orthotopic transplantation. The registry of the International Society for Heart Transplantation, however, has disclosed a strong correlation between duration of graft ischemia and operative (30-day) mortality [12].

Reperfusion damage of an ischemic myocardium has been investigated quite extensively in the past but not in the specific context of cardiac transplantation. Earlier studies have focused on either acute coronary occlusion - involving acute medical or surgical revascularization - or short periods of global ischemia at normothermia or moderate hypothermia in animal experiments. In general, it has been difficult to distinguish sequelae of an ischemic insult from those resulting from reperfusion. The majority of hearts transplanted will regain their normal hemodynamic and electrophysical properties early during reperfusion while on cardiopulmonary bypass. Many of these, however, will lose their sinus rhythm and will need catecholamine support when reperfusion has been completed. Thus, a damaging effect of the reperfusion has to be assumed.

Table 3. Myocardial oxygen consumption ($MVO_2$), lactate concentration in coronary sinus blood, and creatine kinase release (mean ± 1 SEM)

| Time of reperfusion (min) | $MVO_2$ (ml/min per 100 g) | | | | | Lactate (mg/dl) | | | | | Creatine kinase (IU/l) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group A | *n* | Group B | *n* | *P* | Group A | *n* | Group B | *n* | *P* | Group A | *n* | Group B | *n* | *P* |
| 30 | 185 ± 12.6 | 12 | 129 ± 15.5 | 9 | < 0.05 | 287 ± 41.7 | 8 | 102 ± 23.1 | 8 | < 0.0025 | 4203 ± 347 | 6 | 2372 ± 754 | 8 | < 0.05 |
| 60 | 161 ± 19.2 | 10 | 155 ± 28.2 | 6 | NS | | | | | | | | | | |
| 90 | 112 ± 10.9 | 12 | 150 ± 31.4 | 6 | NS | 376 ± 56.0 | 7 | 111 ± 27.7 | 6 | < 0.0025 | 14274 ± 2732 | 5 | 4726 ± 1489 | 6 | < 0.01 |
| 120 | 104 ± 14.4 | 9 | 179 ± 21.2 | 4 | < 0.025 | 323 ± 61.7 | 6 | 115 ± 50.1 | 4 | < 0.025 | 17266 ± 4592 | 4 | 7296 ± 3144 | 4 | < 0.10 |
| 180 | 113 ± 13.9 | 9 | 132 ± 12.6 | 4 | NS | 371 ± 44.6 | 3 | 108 ± 43.8 | 4 | < 0.005 | 30572 ± 7312 | 3 | 15723 ± 7757 | 4 | < 0.15 |

It has been our clinical practice to protect donor hearts from ischemic injury by initial cardioplegic arrest using St. Thomas Hospital cardioplegia. This mode of preservation was applied in each of the 269 hearts transplanted orthotopically at our unit.

Even after very short intervals of global ischemia, unmodified reperfusion will result in reduced myocardial contractility and metabolic imbalance [8, 14, 16]. The same is true for longer periods of regional myocardial ischemia [1, 15, 19] where, in addition, increasing CBF resistance has been demonstrated with ongoing reperfusion [2, 3, 18]. This so-called no reflow phenomenon [10, 13] may be explained by the previous ischemic injury, irrespective of the mode of reperfusion. Clear evidence of the existence of reperfusion injury, however, has been demonstrated for hemorrhagic extension of an infarcted area with reperfusion [4, 6, 7, 9].

In a recent series of experiments, Buckberg et al. [5] have conclusively demonstrated the beneficial effects of controlled reperfusion following regional myocardial ischemia. Modification of the composition of the reperfusate, as well as control of reperfusion conditions, resulted in increased myocardial mechanical and metabolic function. Their method of reperfusing an ischemic myocardium by substrate-enriched WBC was, therefore, applied to porcine hearts submitted to 3 h of cold global ischemia, mimicking the clinical situation of distant heart procurement for transplantation.

In the present study, the reperfusate consisted of warm donor blood, modified by the addition of glucose, potassium, amino acids, bicarbonate, and citrate-phosphate-dextrose, the composition used by the UCLA group [5] (see Table 1). No antioxidants (coenzyme Q10) or calcium channel blockers were added. These agents are said to produce additional, specific effects with respect to amelioration of reperfusion injury and should, thus, be studied separately. Oxygen-free radical scavengers (superoxide dismutase and catalase) have also been investigated earlier by our group in a very similar set of experiments [11].

Porcine hearts were chosen for this investigation since their anatomy and susceptibility to ischemia resemble those of the human heart more closely than a canine or murine myocardium. It was our intention to study the effects of reperfusion injury in donor hearts independent of the recipient's immune response. Therefore, an isolated heart model was developed that would allow for hemodynamic and metabolic studies of the donor heart under defined conditions. The controlled nature of the experimental design, using a blood-primed extracorporeal circuit and submitting the hearts to both working and nonworking conditions, renders this model superior to heterotopic transplants, where only passive function of donor hearts can be anticipated. The results of systolic left ventricular function, as obtained in this study, could clearly differentiate the quality of myocardial protection in the two groups investigated. During both retrograde and orthograde perfusion, LVDP and maximum dp/dt showed consistently higher values in hearts reperfused with WBC. Therefore, despite the limited number of hemodynamic parameters, controlled reperfusion resulted in myocardial mechanical function that was superior to that in control hearts. These findings on left ventricular function confirm the results of previous studies on the influence of WBC following regional myocardial ischemia [5].

Using this model of isolated porcine hearts, analysis of myocardial contractility demonstrated an impressive decline in both groups of hearts in the working mode. This effect is thought to be the result of the maximum stress applied to the hearts. An increasing number of organs had to be excluded after more than 60 min of reperfusion due to myocardial failure (systolic left ventricular pressure < 75 mm Hg). Thus, only a small proportion of hearts could be followed for the scheduled reperfusion time of 3 h.

In addition to the positive effect on left ventricular function, a second important finding was made with respect to CBF measurements. Beyond 60 min of reperfusion, CBF data were consistently (though not significantly) higher in the WBC group. Thereafter, these values started to decrease in the control group, while a further increase was noted in the study group. Impaired CBF following prolonged

periods of ischemia has been repeatedly shown in animal experiments [2, 3, 18]. In our investigation, a comparable pressure gradient was maintained across the coronary vascular bed (constant diastolic aortic pressure, right atrial pressure of zero). Thus, a decrease in myocardial wall tension may have caused the higher CBF values in the study group, suggesting improved diastolic function in these hearts. $MVO_2$ was enhanced in the WBC group only beyond the 1st h of reperfusion. During the remaining study period, increased utilization of oxygen supports the results of the hemodynamic measurements. The same applies for the data on lactate and creatine kinase levels in the CS blood, both of which were significantly higher in control hearts. Thus, anaerobic metabolism and cell injury could be considerably reduced by applying WBC. These findings, again, are comparable with those of Buckberg et al. [5], who also demonstrated significant improvement in aerobic myocardial metabolism following controlled reperfusion after regional ischemia. Similar findings were also made by Teoh et al. after the use of terminal WBC in clinical cases of coronary bypass grafting [17].

This study suggests that WBC, when applied to hearts following medium-term cold global ischemia, enhances left ventricular metabolic and mechanical function. Data were collected in a difficult experimental model using an extremely sensitive species, the pig. Despite the small number of hearts available for analysis at the end of the experiment, a clear statistical difference could be obtained in favor of the study group (group B) during the first 2 h of reperfusion. At present, a prospective, randomized, clinical trial is being performed in donor hearts transplanted after more than 3 h of cold ischemia. Initial results confirm a benefit of the method of controlled reperfusion in cardiac transplantation.

## References


1. Althaus U, Gurtner HP, Baur H, Hamburger S, Roos B (1977) Consequences of myocardial reperfusion following temporary coronary occlusion in pigs: effects on morphologic, biochemical, and hemodynamic findings. Eur J Clin Invest 7: 437-442
2. Beller GA, Smith TW, Hood WB (1974) Altered regional myocardial blood flow following coronary reperfusion in acute myocardial ischemia. Clin Res 22: 262A-271
3. Bloor CM, White FC (1975) Coronary artery reperfusion: effects of occlusion duration on reactive hyperemia responses. Basic Res Cardiol 70: 148-152
4. Bresnahan GF, Roberts R, Shell WE, Ross J, Sobel BE (1974) Deleterious effects due to hemorrhage after myocardial reperfusion. Am J Cardiol 33: 82-97
5. Buckberg GD et al. (1986) Studies of controlled reperfusion after ischemia. A series of experimental and clinical observations from the Division of Thoracic Surgery, UCLA School of Medicine, Los Angeles, California. J Thorac Cardiovasc Surg 92: 483-648
6. Capone RJ, Most AS (1978) Myocardial hemorrhage after coronary reperfusion in pigs. Am J Cardiol 41: 259-263
7. DeLoche A, Fabiani JN, Camilleri JP, Joseph D, Schlumberger M, Carpentier A (1976) Revascularization de l'infarctus du myocarde experimental du rat. Etude histologique, histozymologique et electrocardiographique. Coeur Med Interne 15: 407-413
8. Gaudiani VA, Smith JH, Epstein SE (1978) Alterations in regional contractility following cardiopulmonary bypass with intraoperative ischemia. J Thorac Cardiovasc Surg 76: 70-78
9. Hofmann M, Genth K, Schaper W (1975) The influence of reperfusion on infarct size after experimental coronary artery occlusion. Basic Res Cardiol 75: 572-579
10. Jennings RB, Reimer KA (1983) Factors involved in salvaging ischemic myocardium. Effect of reperfusion of arterial blood. Circulation 68 [Suppl 1]: 25-41
11. Jurmann J, Schäfers HJ, Dammenhayn L, Haverich A (1988) Oxygen free radical scavengers for amelioration of reperfusion damage in heart transplantation. J Thorac Cardiovasc Surg 95: 368-377
12. Kaye MP (1987) The registry of the International Society for Heart Transplantation: fourth official report - 1987. J Heart Transplant 6: 63-66
13. Kloner RA, Ganote CHE, Jennings RB (1974) The "no-reflow" phenomenon after temporary coronary occlusion in the dog. J Clin Invest 54: 1496-1499
14. Lazar HL, Buckberg GD, Manganaro AJ, Becker H, Maloney JV Jr (1980) Reversal of ischemic damage with amino acid substrate enhancement during reperfusion. Surgery 80: 702-708
15. Sharma GP, Varley KG, Kim SW, Barwinsky J, Cohen M, Dhalla NS (1975) Alterations in energy metabolism and ultrastructure upon reperfusion of the ischemic myocardium after coronary occlusion. Am J Cardiol 36: 234-241
16. Smith HG, Kent KM, Epstein SE (1978) Contractile damage from reperfusion after transient ischemia in the dog. J Thorac Cardiovasc Surg 75: 452-458
17. Teoh KH, Christakis GT, Weisel RD, Fremes SE, Mickle AG, Romaschin AD, Harding RS, Ivanov J, Madonik M, Ross IM, McLaughlin PR, Baird RJ (1986) Accelerated myocardial metabolic recovery with terminal warm blood cardioplegia. J Thorac Cardiovasc Surg 9: 888-895
18. Walterbusch G, Haverich A, Reuter TH, Borst HG (1982) The effect of coronary flow restriction on the viability of porcine myocardium. Basic Res Cardiol 77: 333-345
19. Wyatt HL, Forrester JS, Tyberg JV, Goldner ST, Logan SE, Parmley WW, Swan HJC (1975) Effect of graded reductions in regional coronary perfusion on regional and total cardiac function. Am J Cardiol 36: 185-193