Transplant Int (1990) 3: 206–211

TRANSPLANT
International

Improved heart and lung preservation in a rat model

G. Wang, J. Reader, J. Hynd, and J. Pepper

Department of Cardiothoracic Surgery & St. George's Hospital Medical School, Blackshaw Road, London SW7 OQT, UK

Received March 23, 1990/Received after revision July 31, 1990/Accepted August 6, 1990

Abstract. We investigated the efficacy of four different preservation solutions in a heart-lung model in the rat. The heart and lungs of the donor were perfused under standardised conditions of temperature, pressure and flow. We studied 4 groups: group 1 received Stanford solution to heart and lung; group 2 received St. Thomas' solution to heart and Papworth solution to lung; group 3 received University of Wisconsin solution to heart and lung; and group 4 received University of Wisconsin solution to heart and Papworth solution to lung. Lung function assessed by arterial pO_2 at a standardised F_IO_2 was significantly better in groups 2 and 4 than in other groups. However, cardiac function as assessed by cardiac output, stroke work index and minute work index was significantly better in group 4 than in any other group. Overall, the combination of solutions in group 4 provided the most effective preservation in this model.

Key words: Preservation, heart-lung, rat – Heart-lung preservation – St. Thomas' solution, in heart-lung preservation – Papworth solution, in heart-lung preservation – UW solution, in heart-lung preservation

Preservation of the heart and lung in the field of transplantation continues to lag behind that of other organs, notably the kidney and liver. While clinical kidney preservation times routinely exceed 24 h and liver 12 h, the "safe" period for clinical heart and lung preservation remains approximately 4 h. Indeed, even within these limits early failure of the new heart is an occasional major problem in experienced transplant units. Current data from the Registry of the International Society for Heart Transplantation shows a progressive increase in 30-day mortality with increasing heart ischaemic time reaching over 18% for hearts ischaemic for more than 4 h [10].

Recently, Wahlberg and colleagues [39] at the University of Wisconsin have developed an "intracellular" preservation solution containing impermeable macromolecules that has led to a marked improvement in liver organ preservation. This study was designed to test, in a randomised manner, four different preservation solutions, two in current clinical use and two employing a simplified version of the original UW "intracellular" solution [7].

Offprint requests to: J. Pepper

Methods

Male inbred Wistar rats were obtained from the Biological Research Facility at St. George's Hospital Medical School, London. Donor rats weighed approximately 200 g and recipient rats weighed approximately 250 g. Both donor and recipient rats were anaesthetised with 0.3 ml of Hypnorm intramuscularly and 0.2 ml diazepam intramuscularly. They were intubated with a size 18FG Medicut intravenous cannula and mechanically ventilated (Harvard rodent ventilation model 683) with 1%–2% ethrane, 40% oxygen, 2.5–3.0 ml tidal volume, a respiratory rate of 40 breaths per minute and positive end-expiratory pressure (PEEP) of 2 cm H_2O.

In all these experiments we have used a simple heart and left lung heterotopic transplant model, recently described by Kaneko and colleagues [22] in the rabbit. There are two important advantages of this model. Firstly, it has a non-working period, which allows the ischaemic grafts to recover, and secondly, both the lung and heart can be studied in a working mode.

Donor preparation

A midline sternotomy incision was made and the thymus removed. The trachea, aorta, right carotid artery, right and left superior vena cavae (SVC) and IVC were isolated. The persistence of right and left superior venae cavae is the normal situation in the rat [12]. A 16-gauge (OD 1.02 mm) intravenous cannula was placed in the ascending aorta via the right carotid artery for delivery of the heart preservation solution. An identical type of cannula was inserted via the IVC, across the tricuspid and pulmonary valves for delivery of lung preservation solution into the pulmonary artery (PA). After systemic heparinisation (1.5 mg heparin per gram body weight) into the right SVC, the left SVC was ligated and the IVC snared around the PA cannula. The right SVC was left open for drainage of heart preservation solution and the left atrial appendage incised for drainage of lung preservation solution.

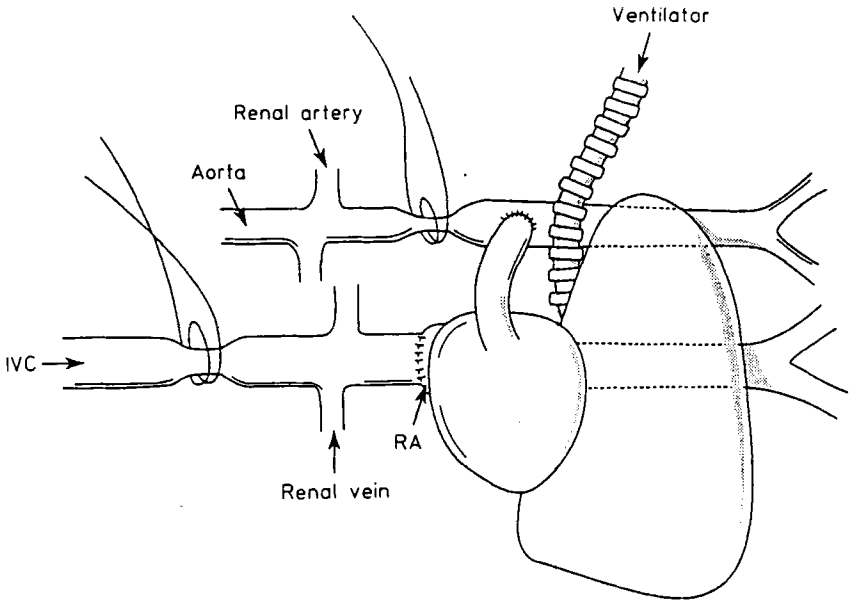

Fig. 1. Experimental arrangement, modified from Kaneko et al. [22]. Ligation of the abdominal aorta below the renal arteries and the inferior vena cava *(IVC)* above the renal veins allowed the donor heart and lung to function in a "working" mode

The aorta was ligated distal to the right carotid artery and 0.2 ml/g of 4°C heart preservation solution infused into the aortic root at 80 mm/Hg for 2 min. The heart normally stopped beating after 30–40 s. Concurrently, the lungs were perfused with 0.6 ml/g of 4°C lung preservation solution by gravity flow from a height of 30 cm above the lung for 2 min. At the end of this time the lungs invariably became uniformly white. During perfusion the respiratory rate was reduced to 20 per minute.

The ascending aorta, left SVC and IVC were divided. The right lung was ligated at the hilum and the entire right lung removed. The trachea was divided four rings above the carina and the heart and lung block placed in storage solution (Ringer's buffered saline) in a refrigerator at 4°C ± 1°C with the temperature monitored using a mercury thermometer.

Recipient preparation

The animals were anaesthetised, intubated and connected to a mechanical ventilator as already described. Body temperature was maintained at 38°C by a warming blanket and monitored by a rectal temperature probe. A laparotomy was performed, the intestines were displaced from the mid-line and wrapped with moist gauze. The infrarenal aorta and IVC were isolated for anastomosis and the infrarenal aorta and suprarenal IVC were isolated for ligation.

Transplant operation

The heart-lung block was removed from the storage solution and placed in the abdomen of the recipient. Anastomoses were made between the donor ascending aorta and the recipient abdominal aorta (end-to-side) and between the donor SVC and recipient infrarenal IVC (end-to-side; Fig. 1). A continuous suture of 10/0 Ethilon was used for both anastomoses. Intravenous cannulae (OD 0.7 mm) were placed into the right atrium (RA) via IVC and into the left ventricle (LV) via the left atrium (LA) for pressure measurement. These were connected via Gould pressure transducers to a Devices 4 channel recorder.

On removal of the clamp, the donor trachea was intubated and connected to a mechanical ventilator. The donor left lung was ventilated with 40% oxygen and 5% carbon dioxide at a tidal volume of 1.8 ml and respiratory rate of 50 per minute and 1 cm H_2O of PEEP. After 1 h of reperfusion of the heart and lung, the grafts were converted from a non-working to a working mode by ligating the infrarenal aorta and the suprarenal IVC. After ligation the preserved heart and left lung provided perfusion to the lower extremities and oxygenation of venous blood. Cardiac output was assessed by a Gould electromagnetic flow probe placed around the abdominal aorta distal to the anastomosis. Blood gas samples were taken from the donor LV and measured in a dedicated ABL_2 acid-base laboratory machine.

Cardiac function determination

Left ventricular function was assessed after 1 h of reperfusion in the non-working mode plus 1 h in the working mode. All measurements were made in triplicate. Stroke work index (SWI in erg/g) was calculated by the following equation:

$$SWI = \frac{(MAP - LVEDP) \cdot CO}{HR \cdot WT} \cdot 1{,}333$$

with MAP = mean aortic pressure (mm Hg), CO = cardiac output (ml/min), HR = heart rate and WT = heart weight (g).

Minute work index (MWI) was calculated in the following way: MWI = SWI · HR expressed in erg/g per minute.

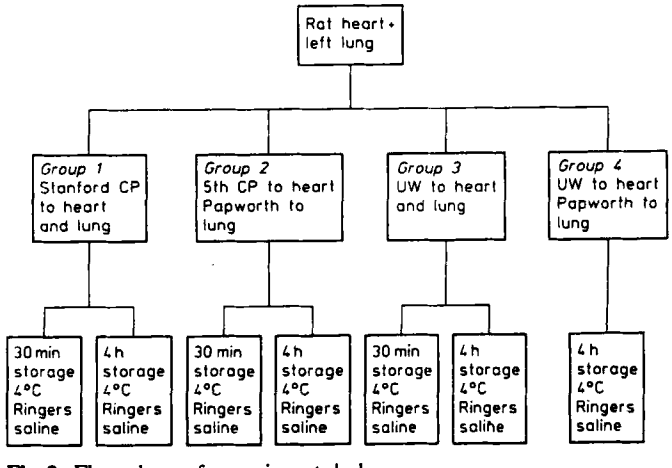

Fig. 2. Flow chart of experimental plan

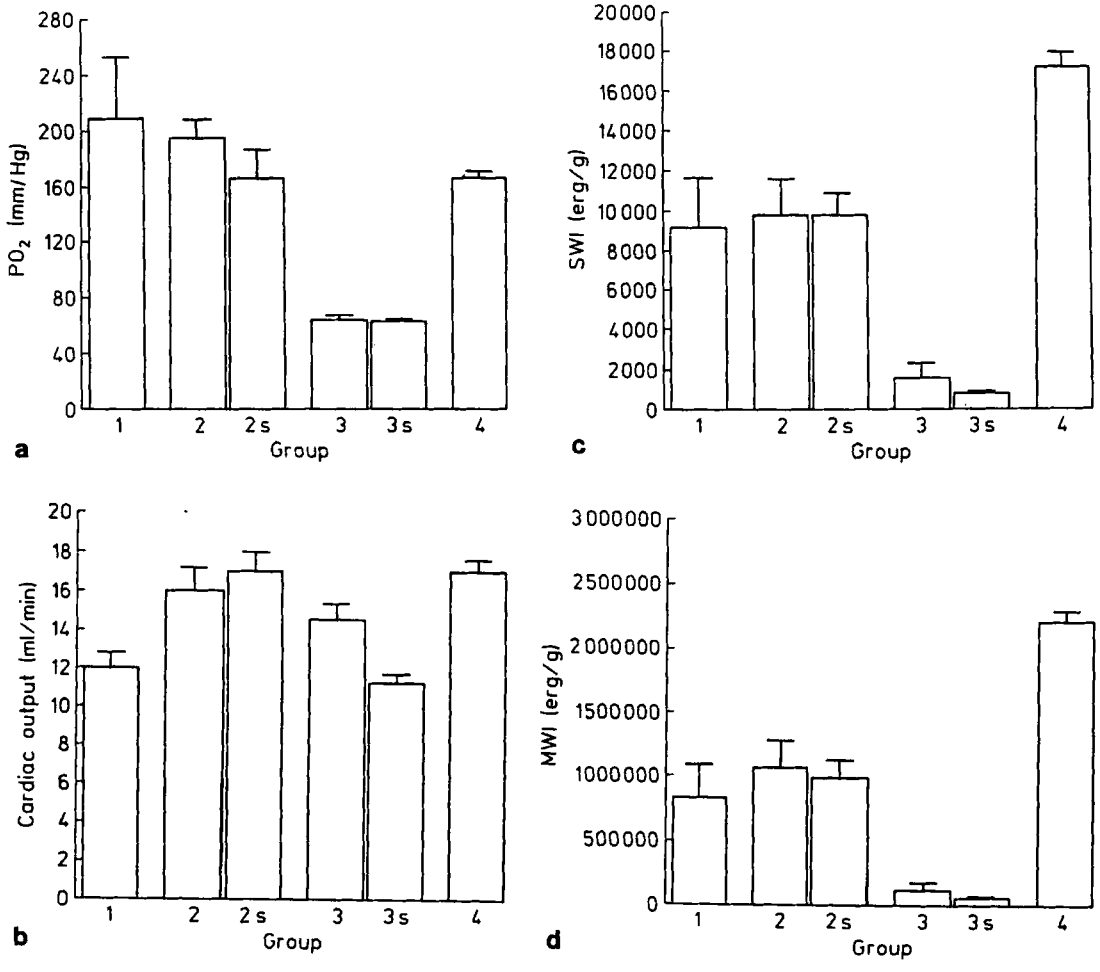

Fig. 3. **a** Arterial pO_2 after 1 h in "working mode" (mean ± SEM). There was a significant difference ($P < 0.01$) between groups 3, 3s and groups 1, 2 and 4. **b** Cardiac output after 1 h in "working mode" (mean ± SEM). Groups 2 and 4 showed a significant difference from groups 1 and 3 ($P < 0.01$), but no significant difference was found between groups 2 and 2s. **c** Stroke work index *(SWI)* after 1 h in "working mode" (mean ± SEM). Group 4's SWI was significantly greater than that of any other group ($P < .001$), and those of groups 1, 2 and 2s were greater than those of 3 and 3s ($P < 0.01$). **d** Minute work index (MWI) after 1 h in "working mode" (mean ± SEM). Group 4's MWI was significantly greater than that of any other group ($P < 0.01$), and those of groups 1, 2 and 2s were greater than those of 3 and 3s ($P < 0.01$)

Statistical analysis

Results are expressed as the mean ± the standard error of the mean. Multiple group comparisons were performed by a one-way analysis of variance. Two group comparisons were made using the unpaired, two-tailed Student's *t*-test. Differences were considered significant at the 5% level: $P \leq 0.05$.

Experimental design

The design is outlined in Fig. 2. Stanford cardioplegia solution (group 1) was compared with St. Thomas' Hospital cardioplegia to heart and Papworth solution to lung (group 2). These groups were also compared with UW solution to heart and lung (group 3) and UW solution to heart and Papworth solution to lung (group 4). Control groups were established in groups 1, 2 and 3 by keeping the storage period to the minimum time required to perform the vascular anastomoses (approximately 30 min). Ringer's buffered saline was used as a storage solution in all groups. No control group was studied in group 4 as it was felt that the control groups for 2 and 3 were adequate. The notation subscript s, as in "2s" and "3s", refers to the experimental group that received 4 h of storage. Animals were randomised to the different test solutions. All preservation solutions, including the UW solution, were freshly prepared at the beginning of the experiments.

Results

Functional recovery

The "arterial" pO_2 (measured from the LV of the donor heart) was significantly greater in groups 1, 2 and 4 (242 mm/Hg, 238 mm/Hg and 238 mm/Hg, respectively) than that in groups 3 and 3s (201 mm/Hg and 185 mm/Hg, respectively; $P < 0.01$; Fig. 3 a). These measurements were made after 1 h in the working mode at F_IO_2 0.4%. There was no significant difference between groups 1, 2 and 4. No recovery was obtained in either the heart or the left lung after 4 h of storage with Stanford solution. Therefore, no data appear for this subgroup in Fig. 3 a–d.

Cardiac output was significantly greater after 1 h in the working mode in groups 2 and 4 (17.1 ml/min and 17.0 ml/min) compared to groups 1 and 3 (12.0 ml/min and 11.2 ml/min; $P < 0.01$). There was also a significant difference between the subgroups 3 and 3s (14.5 ml/min and 11.2 ml/min; $P < 0.05$). Stroke work index was calculated for all groups as shown in Fig. 3c. Group 3 showed markedly inferior results to the other groups, but interestingly the SWI in group 4 was significantly greater than that in all other groups ($P = 0.001$). The minute work index (MWI erg/g per minute) shown in Fig. 3d revealed significantly greater values for group 4 than for any of the other groups. The mean MWI for group 3s was significantly less than for any other group.

Discussion

This study was designed to test different flush solutions for simple hypothermic preservation of the heart and lung in an acute animal model. Although orthotopic heart and lung preservation in subhuman primates is an ideal model, it is too costly to be used for testing a number of solutions. We have compared the UW solution given as cardioplegia to the heart with the commonly used techniques of Stanford cardioplegia and St. Thomas' Hospital No 1 cardioplegia. With respect to the heart, we found markedly enhanced functional recovery with the UW solution, as reflected by the stroke work index and minute work index. In contrast, UW solution had a deleterious effect on lung recovery, as shown by the markedly reduced arterial pO_2 levels. The most efficacious combination for heart-lung preservation was UW solution flush to the heart and Papworth flush to the lung.

The Stanford solution is typical of so-called extracellular cardioplegic solutions and is widely used in North America. As shown in Table 1, it is an aqueous solution containing a moderate concentration of potassium chloride as the cardioplegic agent, sodium bicarbonate as a buffer, glucose as an osmotic agent and mannitol for its osmotic properties and possibly as a free-radical scavenger [23, 24]. The St. Thomas' Hospital solution was introduced into clinical practice in 1975 [4]. Its formulation was based on the idea of deviating as little as possible from the normal extracellular ionic composition and, in particular, to minimise the amount of potassium used to ensure rapid and complete diastolic arrest. Unlike the Stanford solution, the St. Thomas' solution resembles extracellular fluid more closely by containing small amounts of calcium and magnesium and a similar osmolality.

Table 1. Composition of organ flush solutions

	Stanford	St. Thomas' No. 1	Papworth	UW
Na (m*M*/l)	125	144	144	30
K (m*M*/l)	18	20	20	120
Cl (m*M*/l)	98	–	–	–
HCO_3 (mm/l)	45	–	–	–
Dextrose (g/l)	50	–	–	–
Mannitol (g/l)	25	–	20	–
Salt-poor albumin (g/l)	–	–	40	–
Ca (m*M*/l)	–	2.4	–	–
Mg (m*M*/l)	–	16	–	5
SO_4 (m*M*/l)	–	–	–	5
PO_4 (m*M*/l)	–	–	–	25
Lactobionate (m*M*/l)	–	–	–	100
Raffinose (m*M*/l)	–	–	–	30
Adenosine (m*M*/l)	–	–	–	5
Gluthathione (m*M*/l)	–	–	–	3
Allopurinol (m*M*/l)	–	–	–	1
HES (g/l)	–	–	–	50
Procaine hydrochloride (mmol/l)	–	1.0	–	–
pH	7.8	7.0	–	7.4
Osmolality (mosmol)	470	320	–	320

The University of Wisconsin solution was developed by Wahlberg and colleagues for pancreas preservation [39]. It is an "intracellular" high-potassium (120 m*M*/l) solution containing the macromolecules raffinose (MW 594) and lactobionate (MW 358) to reduce hypothermia-induced cell swelling. The solution also contains substances of potential benefit: hydroxyethyl starch as a colloid [38], allopurinol to prevent xanthine-oxidase-mediated oxygen-derived free radical release, glutathione as a reducing agent, adenosine as a precursor for synthesis of adenine nucleotides [39], magnesium for membrane stabilisation and phosphate as a buffer. The solution contains no substrates for glycolysis or oxidative phosphorylation, nor is it supplemented with Krebs' cycle intermediates that have been shown to improve myocardial recovery [1, 31, 36].

In animal studies the UW solution has been found to be highly efficacious for cold preservation of the pancreas [39], kidney [27], and liver [18]. Swanson and colleagues [35] have recently reported improved canine 5- and 12-h heart preservation using the UW cardioplegic and storage solution as compared to Stanford cardioplegia and storage.

Because of these encouraging findings, many transplantation centres have switched their clinical protocols to the use of UW solution. Although no randomised prospective trials have been published, historically controlled series suggest an important improvement in both kidney [17] and especially liver [21] graft function following preservation with UW solution. There have been two cautionary reports [21, 22] that indicate the occurrence of bradyarrhythmias following perfusion of kidneys preserved with UW solution. This side effect has been attributed to adenosine leakage from the kidney. Adenosine, apart from being a potent vasodilator, is well known as a cause of bradyarrhythmia. Clinical heart preservation with UW solution has not yet been reported.

The composition of the UW solution is complex and the mechanisms by which it provides superior preservation remain speculative. Jamieson et al. used the buffer-perfused rabbit liver to ascertain the essential components of the UW solution [18]. He concluded that the essential components of UW solution for liver preservation are lactobionate, raffinose and, perhaps, glutathione.

With hypothermia the transmembrane sodium-potassium ATP-ase becomes inactive, allowing a flux of potassium out of the cell and sodium chloride and water into the cell [3]. The use of a preservation solution more closely resembling intracellular potassium and sodium

concentrations reduces the gradient for such potentially deleterious transmembrane ion fluxes. The concept of an "intracellular" organ preservation solution is an old one, originally introduced by Collins et al. [6] and Sacks et al. [32] for kidney preservation and by Griepp et al. [13] and by Reitz et al. [30] for heart preservation. All these investigators found "intracellular" solutions to improve organ preservation. Davtyan et al. [7] have recently reported the use of modified Sacks' solution for neonatal heart storage. They found that when combined with substrate-enhanced, cardioplegic blood reperfusion there was significantly improved recovery. However, there is as yet no unanimity about the superiority of intracellular solutions.

The potential benefit of allopurinol in the UW solution is controversial. Its incorporation is based on the finding that xanthine oxidase releases oxygen-derived free radicals [24]. These highly reactive molecules, which are released during perfusion, cause widespread cellular injury. Allopurinol, a specific inhibitor of xanthine oxidase, has been shown to reduce myocardial reperfusion injury in a variety of animal models [2, 5, 16, 31]. However, the concentration of xanthine oxidase has been reported as undetectable in both porcine and human hearts [8, 28], which makes its clinical relevance at least questionable.

A property of UW solution that is probably important is the presence of magnesium. The reasons for adding magnesium to a cardioplegic solution include "membrane stabilisation", reduction in transmembrane magnesium efflux and the effect magnesium has on reducing calcium influx [20]. In a dose-response study using the St. Thomas' Hospital solution, a magnesium concentration of 16 mmol/l was found to be optimal [15]. Others have also found magnesium to be helpful [9].

There is currently no consensus regarding lung preservation. In particular it is not clear whether a preservation solution should have an intracellular or extracellular ionic composition or whether the solution should be colloid or crystalloid [11, 37]. The cold-preserved lung appears to suffer injury upon reperfusion. The addition of a free radical scavenger such as allopurinol or mannitol to any preservation solution may improve lung function by reducing the reperfusion injury [25].

The presence of adenosine in UW solution could be an important disadvantage for lung preservation. Adenosine may cause the formation of oxygen-free radicals by being converted to hypoxanthine. The lung may also require a higher concentration of oncotic agents to prevent fluid from entering the alveolar space. Papworth solution contains a large amount of albumin and appears to be successful clinically in preventing this latter problem [40].

Experience in myocardial preservation has suggested that reperfusion injury is based on Ca^{++} influx into the cell caused by a change in the permeability of the cell membrane. Recently, it has been shown that the calcium channel blocker verapamil can reduce tissue damage caused by ischaemia in a normothermic model [14]. This clearly opens the door to further pharmacological manipulation of preservation solutions.

Acknowledgement. This study was supported by a grant from St. George's Hospital Special Trustees.

References

1. Allen BS, Okamoto F, Buckberg GD (1986) Studies of controlled reperfusion after ischaemia XV. Immediate functional recovery after six hours of regional ischaemia by careful control of conditions of reperfusion and composition of reperfusate. J Thorac Cardiovasc Surg 92: 621–635
2. Bando K, Tage M, Teramoto S (1988) Prevention of free radical-induced myocardial injury by allopurinol. Experimental study in cardiac preservation and transplantation. J Thorac Cardiovasc Surg 95: 465–473
3. Belzer FO (1988) Principles of solid organ preservation by cold storage. Transplantation 45: 673–676
4. Braimbridge MV, Ghayer J, Bitensky L, Hearse DJ, Jynge P, Cankovic-Darracott S (1977) Cold cardioplegia or continuous coronary perfusion? J Thorac Cardiovasc Surg 74: 900–906
5. Chambers DE, Parks DA, Patterson G (1985) Xanthine oxidase as a source of free-radical damage in myocardial ischaemia. J Mol Cell Cardiol 17: 145–152
6. Collins GM, Hartley LCJ, Clunie GJA (1972) Kidney preservation for transportation. Experimental analysis of optimal perfusate composition. Br J Surg 59: 187–189
7. Davtyan L, Corno A, Laks H (1988) Long-term neonatal heart preservation. J Thorac Cardiovasc Surg 96: 44–53
8. Eddy LJ, Stewart JR, Jones HP, Engerson TD, McCord JM, Downey JM (1987) Free radical-producing enzyme, xanthine oxidase, is undetectable in human hearts. Am J Physiol 253: H709–711
9. Engleman RM, Rousou JH, Leneshow S, Dobbs WA (1985) A clinical comparison of potassium and magnesium – potassium crystalloid cardioplegia: metabolic considerations. J Cardiovasc Surg 26: 343–350
10. Fragomeni LS, Kaye MP (1988) The registry of the international society for heart transplantation: fifth official report – 1988. J Heart Transplant 7: 249–253
11. Fujimura S, Handa M, Kondo T, Ichinose T, Shirairski Y, Nakada T (1987) Successful 48 hour simple hypothermic preservation of canine lung transplants. Transplant Proc 19: 1334
12. Greene EC (1963) Anatomy of the rat. Hofner, New York London, p 224
13. Griepp RB, Stinson EB, Angell WW, Dong E, Shumway NE (1974) Hypothermic preservation of the canine heart. Transplant Proc 6: 315–318
14. Hachida M, Morton DL (1988) The protection of ischaemic lung with verapamil and hydralazine. J Thorac Cardiovasc Surg 95: 178–183
15. Hearse DJ, Stewart A, Braimbridge MV (1978) Myocardial protection during ischaemic cardiac arrest – the importance of magnesium in cardioplegic ionfusates. J Thorac Cardiovasc Surg 75: 877–883
16. Hearse DJ, Manning AS, Downey JM, Yellon DM (1986) Xanthine oxidase: a critical mediator of myocardial injury during ischaemia and reperfusion? Acta Physiol Scand [Suppl] 548: 65–78
17. Henry ML, Sommer BG, Ferguson RM (1988) Improved immediate function of renal allografts with Belzer perfusate. Transplantation 45: 73–75
18. Jamieson NV, Lindell S, Sundberg R, Southard JH, Belzer FO (1988) An analysis of the components in UW solution using the isolated perfused rabbit liver. Transplantation 46: 512–516
19. Jamieson NV, Sundberg R, Lindell S (1988) Successful 24- to 30-hour preservation of the canine liver: a preliminary report. Transplant Proc 20: 945–947
20. Jamieson WRE (1987) Cardioplegic additives: the importance of optimisation of dose. In: Roberts AJ (ed) Myocardial protection in cardiac surgery. Dekker, New York, pp 321–362

21. Kalayoglu M, Sollinger HW, Belzer FO (1988) Extended preservation of the liver for clinical transplantation. Lancet I: 617–619
22. Kaneko M, Perrotta NJ, Copeland JG (1988) A simple, working heart and lung transplant model for assessing preservation methods in the rabbit. Transplantation 46: 624–626
23. Magovern GJ, Bolling SF, Casale AS, Bulkley BH, Gardner TJ (1984) The mechanism of mannitol in reducing ischaemic injury: hyperosmolarity or hydroxyl scavenger? Circulation 70 [Suppl 1]: 91–95
24. McCord JM (1985) Oxygen derived free radicals in post-ischaemic tissue injury. N Engl J Med 312: 159–163
25. Paull DE, Keagy BA, Kron EJ, Wilcox BR (1989) Improved lung preservation using a dimethylthiourea flush. J Surg Res 46: 333–338
26. Petsikos D, Ricci MA, Balfour R, Varennes B de, Symes J, Guerraty A (1990) Enhanced 24-hour in vitro heart preservation with adenosine and adenosine monophosphate. J Heart Transplant 9: 114–118
27. Ploeg RJ, Gossers D, McAnulty JF, Southard JH, Belzer FO (1988) Successful 72-hour cold storage of dog kidneys with UW solution. Transplantation 46: 191–196
28. Podzuweit T, Braun W, Muller A, Schaper W (1986) Arrhythmias and infarction in the ischaemic pig heart are not mediated by xanthine oxidase-derived free oxygen radicals. Circulation 74: 346
29. Prien T, Dietl K, Zander J, Hacherberg T, Buchholz B (1989) Bradyarrhythmia with University of Wisconsin preservation solution. Lancet I: 1319–1320
30. Reitz BA, Brody WR, Hickey PR, Michaelis LL (1974) Protection of the heart for 24 hours with intracellular (high K +) solution and hypothermia. Surg Forum 25: 149–151
31. Rousou JA, Engelman RM, Anisimowicz L (1986) Metabolic enhancement of myocardial preservation during cardioplegic arrest. J Thorac Cardiovasc Surg 91: 270–276
32. Sacks SA, Petritsch PH, Kaufman JJ (1974) The use of hyperosmolar perfusates in renal preservation. Transplant Proc 6: 283–288
33. Segel LD, Ensunsa JL (1988) Albumin improves stability and longevity of perfluorochemical-perfused hearts. Am J Physiol 254: H1105–H1112
34. Stewart JR, Crute SL, Loughlin V, Heus ML, Greenfield LJ (1985) Prevention of free radical-induced myocardial reperfusion injury with allopurinol. J Thorac Cardiovasc Surg 90: 68–72
35. Swanson DK, Pasaoglu I, Berkoff HA, Southard JH (1988) Improved heart preservation with UW preservation solution. J Heart Transplant 7: 456–467
36. Teoh KH, Mickle DAG, Weisel RD (1988) Improving myocardial metabolic and functional recovery after cardioplegic arrest. J Thorac Cardiovasc Surg 95: 788–798
37. Toledo-Pereyra LH, Hau T, Simmons RL, Najarian J (1977) Lung preservation techniques. Ann Thorac Surg 23: 487–492
38. Vanrenterghem Y, Roels L, Vandermeersch E, Christiaens R, Gruwez J, Michielsen P (1989) University of Wisconsin preservation solute and bradyarrhythmia. Lancet II: 745
39. Wahlberg JA, Southard JH, Belzer FO (1986) Development of a cold storage solution for pancreas preservation. Cryobiology 23: 477–482
40. Wallwork J, Jones K, Cavarocchi N, Hakim M, Higenbottam M (1987) Distant procurement of lungs for clinical heart-lung transplantation using a single-flush technique. Transplantation 44: 654–658