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Organ preservation solutions: clinical and pharmaceutical aspects

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Systematic research has led to continuing improvements in the solutions and techniques of preserving donated hearts. The persistent shortage of donors means that organs of lower quality are increasingly being accepted and we are turning more and more to machine perfusion of donated hearts.

Introduction

Organ transplantation has become a standard therapy for end stage organ failure. Due to standardised techniques, better immunosuppression and more experience of how to cope with post-transplant complications the outcome of transplantation has improved. As a result, indications for transplantation have broadened, in turn resulting in long recipient waiting lists. Despite many major efforts to increase the donor pool of deceased heart-beating brain dead donors (DBD), the addition of living donor (LD) programmes and the exploration of deceased cardiac death (DCD) donors, the persisting donor shortage remains a key problem in transplantation.

Irrespective of the donor source, all organs that will be transplanted need to be preserved during the time between retrieval and implantation. Maintaining organ viability during preservation is an important prerequisite for successful outcome after transplantation. Currently, most centres in Europe use static cold storage (CS) to preserve organs. This technique requires a rapid vascular flush and wash-out with removal of blood, rapid cooling of the organ, and equilibration between the CS solution and tissue. Over the years several cold storage solutions have been developed. Presently, Histidine-Tryptophan-Ketoglutarate or HTK (Custodial) and University of Wisconsin Cold Storage Solution (Viaspan)

are most commonly used in DBD donors. In the Eurotransplant region the use of HTK is increasing since almost all organs from DCD donors are flushed with and stored in HTK solution due to the lower cost at high volumes.

The CS preservation methods, however, were developed in an era with younger and good quality donor organs [1]. Preservation of DBD kidneys for less than 24 hours generally results in adequate function and graft survival, while preservation times and methods appear to be more critical in the outcome of DCD kidneys, which are associated with inferior graft survival.

Principles of cold storage preservation

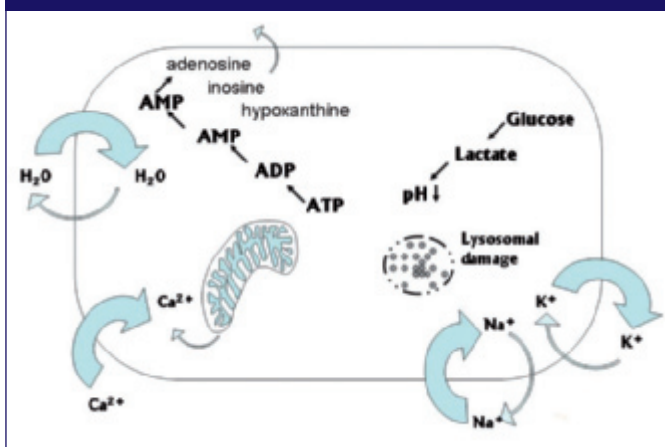
Removal of the organ from the circulatory system leads to disruption of the supply of blood. The absence of oxygen delivery to the cells will rapidly lead to

major metabolic problems. Suppression of metabolism is therefore essential to prolong the time of ischemia the organ can sustain. Reducing the core temperature of the organ below 4°C will result in a reduction of metabolism to 5–8% in the majority of cells and will diminish enzyme activity [2]. As early as 1963, Calne showed that simple cooling of kidneys in ice water preserved function of kidneys for 12 hours: the temperature effect [3]. With a preservation solution, however, cold ischemia times (CIT) can be significantly prolonged and preservation quality improved: the solution effect [4]. Despite the beneficial concept of hypothermia, it causes several unwanted side effects in the preserved organ such as cell swelling, acidosis, and production of radical oxygen species upon reperfusion (see Figure 1).

Composition of clinically used solutions

With the introduction of the first static cold storage solution by Dr Collins in 1969 [5] prolonged preservation of kidneys became clinically feasible. The original Collins solution was modified by the Eurotransplant Foundation in 1976 eliminating magnesium. The Euro-Collins (EC) solution was a simple and cheap intracellular fluid-like preservation solution (see Table 1). Phosphate was used for pH buffering and glucose served as the osmotic agent. However, since the introduction of the University of Wisconsin Cold Storage Solution (UW-CSS)

Figure 1: (Cold) ischaemia induced changes



Negative effects of cold ischemia are breakdown of ATP, acidosis, release of lysosomal enzymes, increased Na⁺ and Ca²⁺ entrance into the cell and subsequent cell swelling.

most centres have changed to this solution. A randomised clinical trial comparing EC with UW-CSS showed that there was significantly less delay in graft function in the UW-CSS group (23% vs. 33%). One year graft survival was also found to be significantly higher in the UW-CSS group [6]. As a result of this study EC is no longer the preferred solution for kidney preservation in Europe.

University of Wisconsin solution

Continuing and systematic research by Belzer and Southard in the 1980s led to the development of the University of Wisconsin Solution and its clinical introduction in 1987. Metabolically inert substrates such as lactobionate and raffinose serve as osmotic agents (see Table 1). Hydroxyethyl starch is used as a colloid. Scavengers (glutathione, allopurinol) and an ATP precursor (adenosine) have been added to the solution. To date, UW-CSS is considered the gold standard preservation solution for kidney, liver, pancreas and small bowel [6-9].

Histidine-Tryptophan-Ketoglutarate (HTK) solution

HTK solution was initially introduced as a cardioplegic solution in open heart surgery by Bretschneider in the 1970s [10]. The basic design of the solution consists of a very potent buffer, histidine, combined with two amino acids (see Table 1). Tryptophan serves as membrane stabilizer and anti-oxidant while ketoglutarate acts as substrate for anaerobic metabolism during preservation. HTK has a low viscosity and to achieve complete tissue equilibration according to Bretschneider, high volumes (~15 L) have to be rinsed through the organs at low flow rates. A multi-centre randomised prospective trial comparing UW-CSS versus HTK in kidney preservation showed equal results in terms of the incidence of delayed graft function (33% vs. 33%) [7]. For prolonged cold storage times with HTK (>24 hours), however, little data is available. One single-centre study reported a significantly higher incidence of delayed graft function of 50% for HTK

Table 1: Composition of major cold storage organ preservation solutions

	EC	UW	HTK
Colloids			
Hydroxyethyl starch, g/L	-	50	-
Osmotic agents that do not pass semipermeable membranes (mM)			
Glucose	195	-	-
Histidine	-	-	198
Mannitol	-	-	38
Lactobionate	-	100	-
Raffinose	-	30	-
Buffers (mM)			
K ₂ HPO ₄	15	-	-
KH ₂ PO ₄	42.5	25	-
NaHCO ₃	10	-	-
Histidine	-	-	198
Electrolytes (mM)			
Sodium	10	25	15
Potassium	115	120	9
Chloride	-	20	32
Calcium	-	-	0.0015
Magnesium	-	-	4
Magnesium sulphate	-	5	-
Radical Oxygen Species scavengers (mM)			
Glutathione	-	3	-
Allopurinol	-	1	-
Tryptophan	-	-	2
Mannitol	-	-	38
Additives (mM)			
Adenosine	-	5	-
Ketoglutarate	-	-	1

EC: Euro-Collins, UW: University of Wisconsin Cold Storage Solution, HTK: Histidine-Tryptophan Ketoglutarate

versus 24% for UW-CSS preserved kidneys when CIT was over 24 hours [11]. The opposite was reported in a more recent study, with a delayed graft function rate of 16% after HTK preservation versus 56% after UW-CSS [12]. Direct comparison of these conflicting findings is impossible due to different definitions of delayed graft function in the studies.

Preservation by hypothermic machine perfusion

In the late 1960s, hypothermic machine perfusion (HMP) developed by Dr FO Belzer was used by a larger number of centres in the US to preserve kidneys as it was considered the best and only way to transport organs from the donor to the recipient centre. Belzer and co-workers were able to preserve canine kidneys for up to 72 hours using the HMP technique [13] and introduced the HMP technique clinically one year later [14].

With the introduction of an 'effective' CS preservation solution such as EC, the number of kidneys preserved by machine decreased. In the US about 10% of kidneys are nowadays preserved by HMP. In recent years a small increase can be observed presumably as it has become generally accepted that kidneys from DCD donors are better preserved using HMP.

Although modern HMP systems are smaller, lighter and more sophisticated than the original machine used by Belzer (see Figure 2), the principles of HMP have not changed.

Machine perfusion generates a controlled continuous or pulsatile recirculating flow of the preservation solution at 0–4°C. This continuous flow allows complete perfusion of the organ promoting a complete wash-out of blood and subsequent tissue

Figure 2: Professor Belzer with the first 'transportable' Machine Perfusion system



equilibration with preservation solution. Beneficial effects claimed on behalf of machine perfusion are a low incidence of delayed graft function, the possibility of online viability assessment, ability to provide metabolic support during perfusion and the potential to add pharmacological agents to the perfusate.

In kidney preservation, both in animal experiments and in historical controlled clinical studies, HMP has been demonstrated to provide better early graft function compared to static CS [15]. In addition, when kidneys derived from extended, marginal or non-heart-beating donors were analysed, HMP was found to be beneficial [14, 16]. Despite all experimental and small clinical studies, the definitive answer to what extent MP is superior to CS was not given until recently. Within the Eurotransplant setting, a randomised controlled trial was published recently in which the superior effect of machine perfusion over static cold storage was demonstrated for all kidney donors, in terms of improved graft function and survival [17].

Outlook

Despite the fact that static CS preservation methods have facilitated many transplant programmes all over the world, it appears that the increasing challenge of maintaining viability in marginal or extended criteria donor organs has now touched the limits of CS preservation. Even with beneficial additives and enriched compositions, static CS, at best, slows down ischemic damage. To further improve organ

viability, a more dynamic preservation method is needed to fulfil metabolic demands of damaged organs better. Many groups therefore are switching gears and are revisiting the possibilities of HMP or investigating the possibilities of (sub)normothermic perfusion of donor organs [18, 19].

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References

1. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation*. 1988;45(4):673-6.
2. Southard JH, Belzer FO. Organ preservation. *Annu Rev Med*. 1995;46:235-47.
3. Calne RY, Pegg DE, Pryse-Davies J, Brown FL. Renal preservation by ice-cooling: an experimental study relating to kidney transplantation from cadavers. *Br Med J*. 1963;2(5358):651-5.
4. McAnulty JF, Reid TW, Waller KR, Murphy CJ. Successful six-day kidney preservation using trophic factor supplemented media and simple cold storage. *Am J Transplant*. 2002; 2(8):712-8.
5. Collins GM, Bravo-Shugarman M, Terasaki PI. Kidney preservation for transportation. Initial perfusion and 30 hours' ice storage. *Lancet*. 1969;2(7632):1219-22.
6. Ploeg RJ, van Bockel JH, Langendijk PT, et al. Effect of preservation solution on results of cadaveric kidney transplantation. The European Multicentre Study Group. *Lancet*. 1992;340 (8812):129-37.
7. De Boer J, De Meester J, Smits JM, et al. Eurotransplant randomized multicenter kidney graft preservation study comparing HTK with UW and Euro-Collins. *Transpl*

- Int. 1999;12(6): 447-53.
8. Erhard J, Lange R, Scherer R, et al. Comparison of histidine-tryptophan-ketoglutarate (HTK) solution versus University of Wisconsin (UW) solution for organ preservation in human liver transplantation. A prospective, randomized study. *Transpl Int*. 1994;7(3):177-81.
9. Fridell JA, Agarwal A, Milgrom ML, et al. Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution for organ preservation in clinical pancreas transplantation. *Transplantation*. 2004;77(8): 1304-6.
10. Bretschneider HJ. Myocardial protection. *Thorac Cardiovasc Surg*. 1980;28(5):295-302.
11. Roels L, Coosemans W, Donck J, et al. Inferior outcome of cadaveric kidneys preserved for more than 24 hr in histidine-tryptophan-ketoglutarate solution. Leuven Collaborative Group for Transplantation. *Transplantation*. 1998;66 (12):1660-4.
12. Agarwal A, Murdock P, Fridell JA. Comparison of histidine-tryptophan ketoglutarate solution and university of Wisconsin solution in prolonged cold preservation of kidney allografts. *Transplantation*. 2006;81(3):480-2.
13. Belzer FO, Ashby BS, Dunphy JE. 24-hour and 72-hour preservation of canine kidneys. *Lancet*. 1967;2(7515):536-8.
14. Belzer FO, Ashby BS, Gulyassy PF, Powell M. Successful seventeen-hour preservation and transplantation of human-cadaver kidney. *N Engl J Med*. 1968;278(11):608-10.
15. Wight JP, Chilcott JB, Holmes MW, Brewer N. Pulsatile machine perfusion vs. cold storage of kidneys for transplantation: a rapid and systematic review. *Clin Transplant*. 2003;17(4): 293-307.
16. Schold JD, Kaplan B, Howard RJ, et al. Are we frozen in time? Analysis of the utilization and efficacy of pulsatile perfusion in renal transplantation. *Am J Transplant*. 2005;5(7):1681-8.
17. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med*. 2009;360(1):7-19.
18. Guarrera JV, Estevez J, Boykin J, et al. Hypothermic machine perfusion of liver grafts for transplantation: technical development in human discard and miniature swine models. *Transplant Proc*. 2005;37(1):323-5.
19. Van der Plaats A, 't Hart NA, Verkerke GJ, et al. Hypothermic machine preservation in liver transplantation revisited: concepts and criteria in the new millennium. *Ann Biomed Eng*. 2004;32(4):623-31.