

TRANSPLANTATION RÉNALE / KIDNEY TRANSPLANTATION ORGAN PRESERVATION

Milestones and Basic Principles

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ABSTRACT • Current shortage in organ donors led to the expansion of criteria for organ donation placing organ preservation as one cornerstone for successful transplant, graft function and survival. The historical work of Belzer and Collins paved the way for key descriptions of physiopathology of cell ischemia and protection (cytokines roles, oxidative stress, energy shift to lactic acidosis and perfusion pressure changes). Good preservation means immediate recovery of function and prevention of chronic rejection. Two cooling approaches are available : static (SCS : simple cold storage) suitable for all organs, and dynamic (HMP: hypothermic machines perfusion) designed for kidneys and liver. A thorough discussion of historically manufactured and widely sold preservation solutions e.g. EuroCollins, UW solution (Viaspan®) as well as current used solutions e.g. Custodiol® and the new Celsior is available in this review. Obviously, every single organ exhibits different tolerance to warm and cold ischemia depending on its nature and demands after transplant. Future perspectives of organ preservation may be hidden in hibernators which may hold the enigmas of perfect human organ preservation.

Keywords : organ preservation, organ preservation solutions, hypothermic preservation, machine perfusion preservation

RÉSUMÉ • La pénurie actuelle des donneurs d'organes a conduit à l'élargissement sans pareil des critères de don rendant la préservation des organes indispensable pour le succès de la transplantation, la fonction du greffon et sa survie. De nombreux travaux historiques – notamment ceux de Belzer et Collins – ont établi une description détaillée de la physiopathologie de l'ischémie et de la protection cellulaire (rôles des cytokines, le stress oxydatif, l'acidose lactique et les pressions de perfusion). Une bonne préservation signifie une fonction immédiate du greffon et la prévention du rejet chronique. Deux approches de refroidissement sont disponibles : statique (SCS : stockage statique simple) adapté à tous les organes, et dynamique (HMP : machines de perfusion) conçues pour les reins et le foie. Une discussion approfondie des liquides de conservation a porté sur les anciennes et nouvelles solutions comme l'EuroCollins, l'UW (Viaspan®), le Custodiol® et la Celsior. Manifestement, chaque organe diffère par sa tolérance à l'ischémie chaude et froide dépendamment de sa nature et de ses besoins après la greffe. L'avenir de la préservation d'organes se révélera peut-être en dénouant la physiologie des animaux hibernateurs.

INTRODUCTION

Little has changed in the basic principles of organ preservation, since Moukarzel and Benoit's review published in 1989 [1]. The solutions we use nowadays remain similar except for some substrates and impermeant components.

The concept of a solution that mimics intracellular milieu (i.e. rich in K⁺) is no longer true. The invariable aim is always toward increasing tolerance to warm and cold ischemia and preventing reperfusion injury. Other goals were set in extending preservation time to allow safe interval for organ transfer (e.g. travel from country to country), to complete optimal recipient preparation and to perform transplant surgery as an elective or semi-elective procedure.

Many challenges remain, namely preserving organs

with previous injury or from high-risk donors. In this article, we will review history, basic principles, preservation solutions, perfusion machines, and cryopreservation as well as future perspectives of organ preservation.

HISTORY

If we go back to the early years of preservation science, Carrel stated in 1908 “*the perfusion of organs seems necessary to prevent formation of clots*” while proposing the Locke solution, “*a physiologically balanced fluid to prevent clots formation*” [2-3].

In 1963, Calne and Pegg demonstrated that simple surface cooling of the kidney would result in 8 to 12 hours of preservation [4].

No major advances were observed until 1967 when Belzer and his group from San Francisco outlined the cause of increased perfusion pressure with removal of fragile plasma lipoproteins from the perfusion before kidney preservation: a milestone in organ preservation. The resultant product of this innovative process was the cryoprecipitate plasma; at that time, the preservation system included hypothermic pulsatile perfusion [5].

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In 1969, Collins, Bravo-Shugarman and Terasaki revolutionized the field, after publishing their pioneer work on the use of intracellular crystalloid acellular solutions in kidney preservation [6] giving birth to “Collins solution” and later “EuroCollins” (Removal of magnesium phosphate after concerns on magnesium crystal precipitations) [7]. Transplant centers around the world used routinely this solution for almost two decades.

Organ preservation techniques gradually made important advances with numerous changes in preservation solutions and the introduction of perfusion machines. In 1987, a substantial breakthrough by Belzer’s group led to the development of a new preservation solution with successful canine pancreas preservation under hypothermia for 72 hours [8-9]. It was evident that this solution was more effective among other multiorgan solutions available in the transplant field. Named the “University of Wisconsin (UW)” solution, it quickly became the gold standard for preservation of liver grafts in 1989.

But in the next few years, a rival solution emerged: “the histidine-tryptophan-ketoglutarate (HTK)” solution with a very different composition and similar results compared to UW solution but with lower costs [10].

BASIC PRINCIPLES

Good preservation means immediate recovery of function and prevention of chronic rejection. Preservation begins with adequate management of the donor before and during harvesting by maintaining systolic pressure > 100 mmHg and urinary output > 100 ml/h, preventing vasospasm before organ manipulation (especially in kidneys) using mannitol, steroids, chlorpromazine, calcium channel blockers, etc.; it is also important to avoid nephrotoxic drugs, correct acidosis, prevent mechanical injury and keep hematocrit level above 30%.

Human kidney tolerates less than 30 minutes of warm ischemia before irreversible injury. One minute of warm ischemia equals one hour of cold ischemia and the renal cortex specifically has the highest requirements of oxygen (80% of the renal blood flow).

For all these reasons, organ preservation remains a crucial step in the transplant process. At the moment blood circulation stops, cells rapidly switch from aerobic to anaerobic metabolism, consuming almost 20 times more glucose substrates to generate the same amount of adenosine triphosphate (ATP) compared to aerobic conditions. This results in a rapid depletion of intracellular energy substrate stores and the accumulation of toxic metabolites and lactic acid. Consequently, The sodium pump (Na-K ATPase pump), an ATP dependent membrane enzyme stops and cell membrane depolarizes as sodium and calcium ions enter and potassium leaves the cell (3Na^+ v/s 2K^+) [11] coupled with water diffusion into the cell. This leads to severe edema and cell death after many changes in cellular structures:

- Swollen mitochondria will later rupture due to calcium influx and prostaglandin derivatives.

- The nucleus disintegrates from pycnosis to chromosomal margination while lysosomes increase in size than disappear.

Other biocellular changes include ATP breakdown to inosine and xanthine. Endothelin, a peptide with potent vasoconstrictor properties, is another factor that plays a major role in ischemia by inducing vasospasm, delaying recovery of organ function after revascularization [12].

The purpose of organ preservation science is to halt these changes as quick as possible. This is first achieved by **cooling** the organ. Thus, metabolic rate rapidly falls to 10% at 10°C, 5% at 5°C and 3% at around 0°C [1]. It is to note that most enzymes of normothermic animals show a 1.5 to 2-fold decrease in activity for every 10°C decrease in temperature.

While optimal perfusion pressure is unknown but usually preferred to be around 80-100 cmH₂O, The optimal cooling rate is 3.7°C/min since faster cooling leads to severe vasospasm and consequently incomplete washout [1].

Two approaches for organ preservation are currently accepted in clinical practice: static and dynamic.

Simple static cold storage (SCS) is the main method for static storage (kidney, liver, pancreas, heart, lung) using preservation solutions, while hypothermic machines perfusion (HMP) are reserved for dynamic preservation (in kidneys only for the time being). In the latter, energy substrates and oxygen supply for maintaining aerobic metabolism are delivered by vascular perfusion. HMP hold great advantages in preserving organs from marginal donors, with particular interests in the non-heart-beating donor (NHBD) [13].

Organ cooling is accomplished using a cold preservation solution specially formulated to counter the effects of prolonged ischemia and minimize injury associated with reperfusion. These solutions must fulfill certain criteria:

- 1. Minimize hypothermic induced cell edema;
- 2. Prevent intracellular acidosis and intracellular Ca accumulation;
- 3. Must contain substrates to regenerate ATP;
- 4. Counteract effects of oxygen free radicals (OH^- , H^2O^2) especially during reperfusion due to xanthine binding free oxygen (i.e. for lungs and small bowel) [14];
- 5. Contain substantial amounts of physiological buffer (e.g. phosphate, citrate, HCO_3^- , histidine) to maintain pH despite accumulation of lactic acid, as well as large molecules called “impermeants” (e.g. mannitol, glucose, lactobionate or raffinose) to keep an adequate intravascular osmotic potential in the absence of blood proteins, thus minimizing cell swelling.

Historically, the early manufactured fluids had an electrolyte composition mimicking closely intracellular fluid as opposed to extracellular fluid (i.e. high potassium and low sodium concentrations to minimize diffusion). This trend was gradually abandoned as said in the introduction and discussed later.

On the other hand, injury to the transplanted graft seems to occur during reperfusion rather than the ischemic phase. Researchers suggested that some events of reperfusion may result in enhanced immunogenicity of the graft [15]. Oxygen free radicals generated during reperfusion oxidize lipid membranes and cellular proteins and are responsible of reperfusion injury, along with cytokines and nitric oxide that also play a role [16]. Oxygen radicals appear to be also involved in microvascular and parenchymal cell injury of various pathologic disorders associated with organ preservation [17].

Ischemia and reperfusion are associated with marked release of tumor necrosis factor (TNF)- α , interferon- γ , interleukin-1, and interleukin-8 [18]. These cytokines trigger the upregulation of adhesion molecules resulting in leukocyte adherence and platelet plugging after revascularization; graft failure and rejection ensue.

It has been shown in various experimental studies that Allopurinol, an inhibitor of xanthine oxidase, had protective effects if used before the ischemic insult.

Nowadays, the most commonly used solutions are the University of Wisconsin solution (ViaSpan[®]), suitable for kidneys, liver, and pancreas, (with high potassium and low sodium) [19], and the newer Bretschneider HTK solution (Custodiol[®]) with low potassium, initially produced and used for cardioplegia in open heart surgery [20].

Low potassium concentrations improve the solution flushing efficiency by removing vasoconstrictive effects of high potassium.

Hydroxy ethyl starch (HES) conveys no advantages to UW solution when used for cold storage; in fact, it increases the solution's viscosity and the production costs. Later, similar clinical results (if not better) were attained with HES free derivatives preparations compared to the original formulation.

Major concerns have been raised about high potassium solutions in pediatric recipients because of potassium K⁺ load washed out of the graft into the child circulation at the time of unclamping and reperfusion.

"Celsior" another recently developed preservation solution of extracellular-type composition with low-viscosity (due to absence of HES) combines the impermeants and inert osmotic carriers of UW solution (using lactobionate and mannitol) and the strong buffer from HTK solution (using histidine) [21]. "Reduced glutathione" in Celsior, a potent antioxidant, inactivates oxygen reactive species. This solution was specifically designed too for heart transplantation spreading later to other clinical uses in lung, liver, and kidney transplant.

Obviously, every single organ exhibits different tolerance to warm and cold ischemia depending on its nature and demands after transplant. Therefore the heart should resume its function as soon as transplanted and has the shortest tolerance to cold ischemia. It is ideally transplanted within 4 to 6 hours since each additional hour affects survival drastically [22].

Kidneys are susceptible but to a lesser extent and function recovery is earlier if transplanted quickly, ideal-

ly within 18 to 24 h. The liver and pancreas lie in the average time range and are best transplanted within 12 to 15 hours before deleterious effects.

Registry analyses show that duration of cold ischemia is the most significant in determining outcome after transplantation, and probably the only modifiable factor. Besides being a continuous variable, any additional time of cold or warm ischemia is detrimental.

Kidney, liver and pancreas feature different metabolisms, especially when glucose is used as an impermeant in the solution and retrieved by the cell for anaerobic glycolysis (resultant cellular acidosis), influencing distinctively organ preservation [9]. The pronounced effects of glucose use, led to its substitution in the UW solution and the use of raffinose instead, a saccharide of large molecular mass, that does not cross cell membranes in all organs.

In summary, preservation solutions significantly improved organ preservation. While merely slowing down extracorporeal ischemic and hypoxic damage, it is less effective in preventing cellular injury.

The retrospective comparative studies of preservation techniques analyzed outcomes post-transplant. Machine-perfused renal allografts had a lower risk of graft failure in the first year after transplantation and a lower risk of delayed graft function. As a result, these kidneys showed an improved 1-year and 3-year graft survival (Even in expanded criteria donors [ECD] and donation after cardiac death [DCD]), as compared with kidneys preserved by SCS [23-28]. Machine perfusions seem to be cost effective operational systems despite initial budgetary impact [29].

In simple cold storage preservation, approximately 25 to 30% of transplanted kidneys have delayed graft function, as opposed to machine perfusion where the rate is reduced to less than 10%. The perfusate is similar to UW solution, except for the impermeant. In continuous perfusion, lactobionic acid is substituted by gluconate [30].

The latest machine for kidney preservation is "LifePort Transporter" from Organ Recovery Systems based on hypothermic perfusion within an ice-filled cooler. The machine gently perfuses or pumps the kidney with cold liquid solution improving its condition before transplant; organ storage time may be doubled safely with LifePort. This device provides also decisive data to monitor and evaluate kidneys during transport.

The ideal HMP setting has not been yet really defined. However, key questions remain unsolved regarding mandatory oxygenation of preservation fluids to maintain organ viability and efficient function [17].

A lot of work is pursued in the United States and Europe bringing novel advances to fashion perfusion machines suitable for other organs as liver, pancreas, heart and lung. Organ Recovery Systems has developed the "LifePort Liver Transporter" [31] indeed, currently in the process of securing US and European regulatory registrations. HMP for liver is very challenging due to liver dual blood supply via both portal vein and hepatic artery.

FREEZING AND THAWING

Cryopreservation has long fascinated scientists; many attempts were made at cryopreserving organs, sometimes entire bodies, by means of conventional freezing and thawing [32].

Unfortunately, every single organ has different cell types with specific requirements for optimal cryopreservation, rendering recovery of each cell category suboptimal since a single thermal protocol is imposed on the whole organ. Moreover, extracellular ice can cause mechanical damage to the organ structural integrity, in particular the vascular component, where ice is likely to form [32].

Mechanical fractures occur in vitreous solids between ice crystals with thermal traumas at low temperatures. Attachments between cells and with their basement membranes are disrupted and many parts of the organ separate.

Cryopreservation of the entire organ is unachievable with current technology.

Perhaps the most promising approach to cryopreservation of whole organs stands on the process of vitrification [33]: “*the process of transforming aqueous solution into amorphous solid*”. For vitrification to occur at reasonable cooling rates, almost half of the water within the organ is replaced with solute molecules constituting the major operational problem in the vitrification process. Vitrification is not currently used in clinical transplantation.

CONCLUSION

Every transplant surgeon and physician should be familiar with the basic principles of organ preservation in order to better protect the graft influencing positively its future survival. A lot of research and work await transplant academic community in many challenging situations when preserving organs from non-heart-beating organ donors, or high-risk donors with previous injury (e.g. ischemia).

Many questions subsist regarding molecular impacts of organ preservation that need to be addressed in the future. Over the next decade, perfusion technologies at hypothermia and even at normothermic temperatures, will play a paramount role in protecting precious donated organs awaiting grafting and probably during early reperfusion.

It is also expected that further progress in organ cryopreservation to be made over the next years, but actual technology is very far from being implemented into clinical practice.

Hibernators may hold the enigmas of perfect human organ preservation. These animals would hold in extreme cold conditions, surprisingly, without any functional tissue damage after returning to moderate temperatures. The reality of this “cold storage” process in this exceptional member of the animal kingdom, may perhaps improve our understanding of human organ preservation.

Organ preservation story will continue, the future will reveal unprecedented discoveries that will have a big impact on cellular protection.

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