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Improving Organ Preservation: The Trick Is to Keep (Cells) Breathing

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Abbreviations

CS, cold storage

DAMPS, damage-associated molecular patterns

DBD, donation after brain death

DCD, donation after circulatory death

HOPE, hypothermic oxygenated perfusion

OP, organ perfusion

A paradigm shift from "one-size-fits-all" cold storage towards preservation tailored to the needs of a specific organ is essential for the transplantation field to continue to grow. Tailoring organ preservation and resuscitation strategies will allow increasing both number and quality of donor organs (Figure 1, panel a). The rapidly developing domain of dynamic preservation, using organ perfusion technology, illustrates that we can advance beyond the constraints of static cold storage. Organ perfusion may improve preservation and allow viability assessment and active organ repair. Technological progress allows perfusing donor organs at temperatures of our choosing, either in situ (e.g., normothermic regional perfusion in the deceased donor) or ex situ (i.e., machine perfusion). Organ perfusion can be used from procurement to transplantation (continuous perfusion), before, during or after cold storage (Figure 1, panel b). Although we do not yet fully understand which grafts benefit most from what (combination of) perfusion technique(s) and treatments, we now know that active oxygenation is vital, even in the cold.

Donor organs are usually stored in cold hypoxic conditions because preservation solutions are not actively oxygenated, although early work with prolonged organ preservation by Belzer in the 1960s relied on continuous hypothermic oxygenated perfusion (HOPE). Current practice assumes hypothermia reduces metabolism to a level where active oxygenation is not needed. Although metabolism at 4°C is diminished to about 5% of that at body temperature, it is not halted nor is cold ischemia harmless. Increasing cold ischemia time adversely affects early and late posttransplant outcomes, especially in organs donated after circulatory death (DCD) and those donated after brain death (DBD) with comorbidities. Oxygen tensions in the preservation solution decrease with storage time as the dissolved oxygen is used.[1] This results in cessation of mitochondrial oxidative phosphorylation and ATP depletion as forward electron transport at mitochondrial complexes stops.[2] During ischemia electrons are rerouted to succinate, which acts as an electron store in the absence of oxygen.[2] When oxygen is reintroduced at normothermic reperfusion, forward electron transport generates ATP if ischemia was short.

However, in case of too much accumulated mitochondrial succinate, rapid oxidation of the succinate reduces the Coenzyme Q pool and hyperpolarizes the mitochondrial inner membrane.[2] In combination with acidic pH from ischemia these conditions drive reversed electron transfer at complex I, resulting in formation of reactive oxygen species and damage-associated molecular patterns (DAMPS).[2] In response, innate immunity is activated and a sterile inflammation with a maladaptive injury repair initiates alloreactive T and B-cells responses, priming the organ for rejection and fibrosis.[3] Reintroducing oxygen during hypothermia blunts immune responses to ischemia-reperfusion with postreperfusion reduction of DAMPS, endothelial, macrophage, and T-cell activation.[4,5]

Simply adding an oxygen carrier to cold storage seems to improve posttransplant outcomes, likely because the greater diffusion gradient makes the dissolved oxygen more readily available to the tissues.[1,6,7] However, oxygen tensions in the preservation solution still decrease over time as there is no active oxygenation.[1] Furthermore, the solution is not recirculated through the vasculature resulting in an unequal oxygen availability to the tissues which might limit the applicability of this technique.[6]

Hypothermic perfusion techniques overcome the latter limitation but without active oxygenation, the perfusate oxygen tension rapidly declines, implying that oxygenating the perfusate might optimize the technique.[8,9] Preclinical studies have indeed shown increased mitochondrial respiration with HOPE, reaching an equilibrium after about 90 minutes,[10] and increased tissue ATP content that translates into improved graft function.[9,10] In acellular hypothermic conditions, high oxygen concentrations (>600 mmHg) are needed to achieve beneficial effects.[11] Although oxygen is usually provided via a membrane oxygenator added to hypothermic perfusion set-ups, bubbling oxygen through the perfusate can reach similar oxygen tensions.[12] The exact time when to start HOPE is difficult to determine and has not been investigated well. It is also possible this will be different for different organ types. Furthermore,

efficacy of HOPE might differ between donor types as most work on HOPE has been conducted in DCD-models[9] with deeply hypoxic tissues as result of cessation of blood flow before preservation while the nature of predonation hypoxia in DBD donors is more complex, involving autonomic and cytokine storms followed by inflammation.

An increasing body of (pre)clinical evidence shows the benefits of HOPE over cold storage and nonoxygenated hypothermic perfusion in organ transplantation. Low oxygen tensions or absence of oxygen during hypothermic perfusion might explain why preclinical liver studies show no functional benefit of hypothermic perfusion despite prevention of endothelial damage.[10] It might also be one of the reasons why hypothermic perfusion only increases kidney graft survival in DBD and not DCD kidneys, despite improving immediate graft function in kidneys from both donor types.[13]

Logistically easiest to implement is a brief period of HOPE after cold storage. The D-HOPE randomized controlled trial showed that a short period of HOPE after liver cold storage, providing oxygen to hepatic artery and portal vein, reduces the risk of posttransplant clinically significant intrahepatic biliary strictures, postreperfusion syndrome, and early allograft dysfunction in DCDs when compared to cold storage.[14] Results with a short period of HOPE from randomized liver trials in DBD are awaited (NCT01317342 and NCT03124641) with preliminary data pointing toward improved graft function.[15]

Although a brief period of HOPE before implantation improves kidney graft outcomes in rats compared to cold storage, these results have not translated to relevant (pre)clinical settings. Indeed, in a pig DCD transplant model, a short period of HOPE after cold storage did not improve results compared to cold storage alone.[16] In DBD, the POMP randomized controlled trial showed no improvement in graft survival or early outcomes in expanded criteria donor kidneys when cold storage was followed by HOPE compared to cold storage alone, though it must be mentioned that the trial was underpowered because 1-year graft survival rates were

considerably better than anticipated.[17] A mandated minimum of 2 hours of HOPE and a mean actual HOPE-time of 4.67 hours after cold storage did not improve posttransplant outcomes, suggesting that either a longer period of HOPE or an earlier supply of oxygen might be required for HOPE to impact outcomes.

Indeed, in the COMPARE trial, a paired randomized controlled trial of older DCD kidneys, continuous HOPE reduced posttransplant complication rates as well as biopsy-proven acute rejection and improved 1-year graft survival and function compared to continuous hypothermic perfusion without active oxygenation.[18] This apparent difference in clinical findings between liver and kidney is intriguing and unexplained. In kidney, all modalities of oxygen timing during preservation have been studied but only a few preclinical studies have directly compared different approaches. These suggest early oxygen administration is essential.[8,16] Considering the results of the POMP and COMPARE trials, clinical evidence is currently in favor to start HOPE immediately after kidney procurement and until transplantation when logistically possible. Interestingly, only early work on HOPE in liver, again by Belzer, focused on continuous HOPE and it is not known whether continuous HOPE would improve result in liver transplantation further. Feasibility and safety of prolonged heart preservation with HOPE has been shown[19] and a randomized controlled trial comparing continuous HOPE with cold storage is recruiting (NCT03991923). Whether HOPE allows to safely increase cold preservation times is unknown. Until proper studies have been conducted, reducing total cold preservation time as much as possible remains important.

The administration of HOPE only at the beginning of preservation has been contemplated, aimed at avoiding active oxygenation during (air) transport. In a pig transplant model, a brief period of oxygen uploading had similar initial graft function compared to kidneys that had undergone continuous HOPE.[8] In liver, a randomized controlled trial with HOPE uploading is recruiting (NCT03484455). Whether mitochondrial respiration is maintained throughout

perfusion when preoxygenation is applied is unknown but unlikely as perfusate oxygen tensions rapidly drop below the threshold needed for active ATP synthesis when oxygenation is stopped.[8,11] When oxygen uploading, using oxygen bubbling, was combined with intermittent surface oxygenation, initial kidney function in a DCD-pig transplant model was comparable to continuous HOPE.[12] Nevertheless, perfusate oxygen tensions dropped quickly with evidence of reduced mitochondrial respiration in comparison to continuous HOPE.[12] It would therefore be essential to assess longer-term outcomes, and especially the effect on immunity, with this oxygenation strategy.

The mechanisms of the immunosuppressive effect of oxygen, as now described in rodent models and clinical setting,[4,5,18] needs further study. It might be that dendritic cells, the highly specialized, bone marrow-derived antigen-processing and -presenting cells crucial to the induction, integration and regulation of innate and adaptive immunity, play a vital role.[20] Indeed, both DAMPS and hypoxia activate dendritic cells[20] and supply of oxygen during hypothermic preservation, reducing DAMPS and hypoxia at time of reperfusion, might reduce dendritic cell activation, followed by a reduced response of the adaptive immune system.

Oxygen persufflation is another technique to oxygenate organs during cold preservation. Humidified, gaseous oxygen (or oxygen-nitric oxide gas mixture) is delivered directly to the organ via its vasculature after a period of cold storage.[21] The technique is simple, needing only access to the gas mixture, and cheap as no organ perfusion circuits are needed. Most preclinical work in liver, kidney, pancreas, and heart show superiority of persufflation over cold storage in DCD settings.[21] Clinical experience is limited and a recent randomized controlled trial in DBD livers did not show superiority of persufflation over cold storage, though there was some suggestion it might be beneficial for subgroups of high-risk liver grafts.[22] This technique requires further clinical investigation and comparison to HOPE.

A detailed overview of (sub)normothermic perfusion is outside the scope of this paper but the need for active oxygenation is not questioned because organs are metabolically active. Nevertheless, the ideal oxygen tension of the perfusate at (sub)normothermia, with the risk of hyperoxygenation, is not known.

Organ-tailored preservation needs to adhere to the Goldilocks principle. It has to be "just right" for the specific graft. Decades after the pioneering work by Belzer and colleagues, we seem to have come full circle in our understanding that maintaining donor organs requires oxygen, even in the cold, though many questions remain to be answered. One of these is whether HOPE will solely be a preservation strategy or whether viability assessment will be possible, given that the organ maintains low-grade metabolic activity. It is my hope that international collaborations will speed up the development of organ perfusion technologies and our understanding of which type of perfusion, or which combination of perfusion, is best suited when and for which donor and organ type.

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Figure legend

Figure 1. A, Schematic representation of organ-tailored preservation and resuscitation. Organ risk profiles will allow the categorization into either low or high risk for a certain undesirable outcome. These profiles will likely be based upon characteristics of the donor, recipient, procurement, and logistics. When the risk for an adverse outcome is assessed as low, the organ might benefit most from optimal preservation. This could be cold storage or a form of organ perfusion. When the risk is assessed as high, additional quality and viability assessment would be warranted, using organ perfusion techniques as assessment platforms. This quality assessment might reveal injury that can be repaired with the organ then transplanted. When injury is considered too severe for repair by available techniques, the discarded organ could continue to research protocols and perhaps be transplanted via this pathway. B, Schematic representation of the different applications of organ perfusion. (1) continuous organ perfusion (OP) for the entire preservation interval; (2) a brief period of organ perfusion either in the donor (in situ organ perfusion, eg normothermic regional perfusion) or ex situ OP in the donor hospital followed by cold storage (CS) for transportation to the recipient center or organ hub; (3) OP at the recipient center only; (4) an intermittent period of OP which could be executed in an organ hub or at the recipient center, followed by CS until implantation. Adapted from Jochmans et al. (*Curr Opinion Transpl*, 16: 174-9) with permission from the publisher.

Figure 1