Ischemia/Reperfusion during normothermic perfusion

Ischémie-reperfusion en perfusion normothermique ?

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Summary

Introduction: Cold storage of organs for preservation and transplantation is reaching its limits especially with extended criteria for heart beating donors and donation after cardiac death. We will discuss recent findings and perspectives in normothermic kidney preservation.

Methods: A literature review was performed from original articles and syntheses selected by the search engine PubMed. Keywords used were: cold ischemia; warm ischemia, normothermic, organ preservation, preconditioning, organ perfusion.

Results: We identified several ways to improve kidney preservation: Ischemic normothermic preconditioning; Pharmacologic normothermic preconditioning; Ex vivo normothermic reperfusion; Remote ischemic transplantation preconditioning; Ischemic postconditioning.

In clinical practice, only uses of ECMO for organ preconditioning or ex vivo normothermic organ perfusion were used.

Conclusion: Promising experimental and clinical results make challenge cold preservation. The most suitable and physiological method seems to be a normothermic perfusion and conservation with autologous oxygenated blood using Extra Corporeal Membrane Oxygenation or Regional Normothermic Circulation.

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Introduction

Cold storage of organs for preservation and transplantation is reaching its limits especially with extended criteria for heart beating donors and donation after cardiac death. The objective of organ preservation is to ensure the best function after organ transplantation and reperfusion. The method developed until nowadays used cold preservation solutions to flush and preserve organs. These solutions are designed to decrease cellular metabolism, oxygen requirement and tissue injuries [1]. To improve organ preservation, two paths are considered: normothermic conditioning of grafts before or after cold storage and normothermic oxygenated preservation. Thus, we will discuss below recent findings and perspectives in normothermic kidney preservation.

Methods

A literature review was performed from original articles and syntheses already available and selected by the search engine PubMed of the National Library of Medicine. Keywords used for this research were: cold ischemia; warm ischemia, normothermic, organ preservation, preconditioning, organ perfusion. All items were analyzed and identified. The congress abstracts were not included in our analysis.

Results

Cold Ischemia/reperfusion

The Ischemia/Reperfusion (I/R) phenomenon was initially considered as an event surrounding organs procurement. I/R now includes more broadly the state of the donor, with the period of brain death or that of warm ischemia in heart-beating donors. Among the many mechanisms involved in I/R injuries we would like to emphasize the following:

- Increased production of cytokines and overexpression of MHC antigens (MHC) class I and II presenting cells, responsible for an increased immunogenicity.
- The reactive oxygen species, such as free radicals, oxygen ions, and peroxide, activate the innate system of donor and recipient.
- Overexpression of some ligands of Toll-Like Receptors (TLR) such as Heat Shock Proteins (HSP), interact with and activate dendritic TLR4 positive cells. Dendritic cells induce alloimmune adaptive response (acute rejection). Dendritic cells also interact with and activate vascular TLR4 positive cells, thus contributing to the development of chronic lesions.
- The Toll domain of human interleukin-1 induces the activation of dependent on interferon γ (IFN-γ) pathways, which appear to be associated with dendritic cells maturation.
- Mitochondrial edema induced by cold ischemia activates the apoptotic pathway, characterized, which is characterized by a translocation of cytochrome C (with others pro apoptotic proteins), and an increased Bax/Bcl-2 ratio with activation of caspase 3 at the time of reperfusion. The oxidative stress and the thermic shock being the main source of I/R organ injuries, others strategies need to be investigated to improve organ procurement and preservation.

Normothermic kidney conditioning

Ischemic preconditioning

Experimental data

Torras [2], in a rat model, prepared kidney grafts for conventional cold storage by fifteen minutes of warm ischemia and 10 minutes of reperfusion at body temperature. The beneficial effect of preconditioning is related to the local production of Nitric Oxide (NO). This protocol of ischemic preconditioning was
not confirmed in a pig model. Das [3] found that normothermic flushing (with conventional preservation solution as University of Wisconsin) of canine kidneys prior to a hypothermic flush eliminated vasoconstriction effects. Animal models highlighted molecular pathways involved in beneficial effects of normothermic perfusion before cold storage. Brasile [4] demonstrated that warm ischemia could increase intragraft expression of Hem Oxygenase 1 preventing subsequent immune-mediated injury through inhibition of several immune effector functions, including lymphocyte proliferation, tumor necrosis factor and interferon synthesis, and T and natural killer cell mediated cytotoxicity. Warm ischemia could even increase nitric oxyd (NO) production preserving vascular integrity. As a consequence, elevated intragraft HO-1 and NO expression improve organ quality and long-term graft function and survival. Maintaining circulation before procurement is also thought to condition the organs with the up-regulation of adenosine receptors, which may protect against preservation injury.

Clinical practice

The conventional technique of organ procurement begins with in situ placement of an aortic cannula to flush organs with a cold preservation solution. Then the organs are stored in the same cold preservation solution at 4 °C. Some authors tried to improve this technique by maintaining circulation at normal body or room temperature using cardiopulmonary bypass by extracorporeal membrane oxygenation (ECMO) for various periods of time (45 to 90 minutes) before in-situ cooling and conventional cold storage. This technique was designed for Non Heart Beating Donors (NHBD) according to Maastricht classification. Valero opened this path [5]. The incidence of Delayed Graft Function (DGF) and Primary Non Function (PNF) was significantly reduced compared with standard in situ or total body cooling. Many others clinical studies confirmed these results [6-8].

Pharmacologic normothermic preconditioning

Another advantage of normothermic perfusion is to prepare organs for cold storage with various treatments. Erythropoietin (EPO) preconditioning has shown interesting results [9]. EPO mediates preconditioning by limiting the destructive potential of tumor necrosis factor α and other proinflammatory cytokines in an experimental model of rat kidney transplantation. This promising result was not confirmed in a porcine model. Trimetazidine demonstrated its efficiency to prevent I/R injuries in a pig kidney warm ischemia model. Cau [10] reminded, in his study, the various effects of this molecule: decrease in intracellular acidosis, preservation of ATP production, limitation of the inflammatory reaction, and reactive oxygen species generation, prevention of calcium overload, decrease in cell apoptosis. Gok [11] using streptokinase preflush in a clinical model of NHBD, improved the quality of donor’s kidney. Immunosuppressive molecules have an influence on I/R injury in kidneys procured from NHBD. The combination of tacrolimus and micofenolate mofetil significantly reduced TNF-alpha production at the time of reperfusion of the porcine kidney affected by ischemia. Kinsey [12] explored the lymphocyte T regulator (Treg) pathway. As demonstrated previously [13], Tregs suppressed inflammation through contact mediated inhibition of immune cells and through release of soluble mediators such as TGF-beta, IL-10 (which downregulate the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages, enhance B cell survival, proliferation, and antibody production), and adenosine (cytoprotection preventing tissue damage during instances of hypoxemia and ischemia). Pharmacologic stimulation of adenosine receptors (A2AR) with a specific agonist increased the ability of Tregs to suppress kidney I/R injuries in a rat model of warm ischemia.

Except use of streptokinase, pharmacological preconditioning during normothermic perfusion was not explored in clinical conditions.

Kidney resuscitation

Ex vivo normothermic reperfusion

Normothermic preservation is based on the idea that restoring metabolism ex vivo may allow the reversal of the detrimental effects of ischemia prior to transplantation. It allows a period of normothermic perfusion at the end of the ischemic period to « resuscitate » the organ, before transplantation. Various strategies were explored. In pigs, Hosgood [14] and Harper [15] used a period of normothermic perfusion with oxygenated autologous blood to improve post transplantation results. In these studies, the authors showed a lower level of lipid peroxidation suggesting a decreased oxidative stress. With the same study design, Bagul [16] showed normothermic perfusion with oxygenated blood was able to restore depleted ATP levels, to restore acid-base balance and reverse some of the deleterious effects of cold storage such as renal tubular injuries. The same team demonstrated later with the same porcine model, that leucocyte depletion improves renal function during reperfusion. It confirmed that white cells play a key role in organ I/R injuries. Mann, in pig kidneys, added to normothermic continuous perfusion (after 2 hours of cold storage) on a pulsatile machine, a selective endothelin receptor antagonist in a perfusion solution. Results on urine production of the kidneys showed restored renal function after one hour. Hosgood [17] described in 2011 the first ex-vivo normothermic reperfusion after cold storage. With a plasma free red cell-based solution, the transplantation that followed gave good results.

Remote ischemic transplantation preconditioning

Body and organ conditioning before normothermic reperfusion is based on remote ischemic preconditioning after elective abdominal aortic aneurysm repair, which reduces myocardial and renal injury. After study on dogs, Soendergaard [18] demonstrated on pigs that four cycles of 5 min clamping (during the venous anastomosis) of the exposed distal abdominal aorta before final renal graft reperfusion in the recipient can improve initial renal plasma perfusion and glomerular function and decrease DGF. The factors transmitting the protective effect remain unclear. Three possible mechanisms have been proposed involving a neuronal signal transmission, a release of humoral mediators, or the induction of a systemic anti-inflammatory response.
Ischemic postconditioning

In the field of heart transplantation, Zhao [19] demonstrated that short, repeated sequences of ischemia and reperfusion after a prolonged ischemic episode, called ischemic postconditioning, reduce infarct size by about 40% after an ischemic myocardial injury. Szwarz in a mouse model [20], tried to replicate these results. Three cycles of 30 seconds of reperfusion and 30 seconds of ischemia showed renoprotective effects. Molecular pathways were not well understood but the proposed hypotheses were a decreased generation of reactive oxygen species and accumulation of intracellular Ca^{2+}, an activation of the reperfusion injury signaling kinase (RISK) pathway and an anti-apoptotic action by inhibiting the opening of the mitochondria permeability transition pore. Once again, it needed to be assessed in a preclinical model. In experimental kidney transplantation, Serviddio [21] and Jiang [22] demonstrated normothermic postconditioning is an effective strategy to reduce I/R injury. Liu [23] partially highlighted molecular pathways of ischemic postconditioning benefits with the major role of NO. Several other studies since the years 2000 demonstrated benefits of trimetazidine associated with ischemic postconditioning. In addition to repeated normothermic ischemic stimulation, trimetazidine was experimented in a mouse kidney transplantation model. Association of ischemic postconditioning and trimetazidine protects the kidney by suppressing the endoplasmic reticulum and mitochondrial injury. Then this model was transposed in a pig kidney transplantation model with the same results.

Normothermic perfusion and storage

Experimental data

Taking into account that cold storage and oxidative stress are the main mechanisms of renal injuries, it was clearly needed to find new strategies to preserve organs at body temperature with oxygenated solutions. In a rabbit kidney model, Arnaud [24] described normothermic blood perfusion added to cryoprotectants. She concluded that normothermic perfusion can produce informations in real time and has potential utility for the further understanding and treatment of I/R injuries. Impaired oxygen consumption, excessive weight gain and virtual anuria were identified as potentially simple in vitro tests for kidney viability prior to transplantation. Kay [25] found no detrimental effect in porcine kidneys flushed with a novel non-phosphate buffered solution at 32 °C. The study showed that this oxygenated solution could sufficiently maintain viability in flushed porcine kidneys and then statically stored for 2 h at 32 °C. Metcalf [26] compared hypothermic and normothermic pulsatile preservation of paired porcine kidneys during 16 hours. For normothermic perfusion, the solution used was oxygenated autologous blood supplemented by infusions of nutrients and colloid. The ability for kidneys to concentrate creatinine and withhold sodium was significantly better for those preserved at body temperature. These promising results have to be confirmed in a transplantation model. More recently Iwai [27] studied normothermic storage with an extracellular-type solution containing trehalose. Once again, interesting results were observed and the author concluded that normothermic continuous perfusion is able to prevent reperfusion injury due to temperature-dependent tissue edema. Nevertheless, these results were found in a rat model and need to be more relevant to clinical practice. Brasile [28], in the same study reported above, described a totally new concept of normothermic preservation. They called this new approach the acellular near-normothermic exsanguineous metabolic support (EMS). Their purpose was to preserve blood vessel wall function. Cellular metabolism could be adequately supported for up to 48 h at near-normothermic temperatures with stable oxidative metabolism and perfusion characteristics. Human kidneys used in this study could not be transplanted so their function were compared in parallel to canine kidney transplants which were transplanted 24 or 48 hours after procurement. Results were very promising and demonstrated that it is feasible to maintain intact human kidneys during an acellular perfusion with a cell culture-like medium administered for 48 h at 32 °C.

Limitations of normothermic preservation

Logistics

Normothermic perfusion and preservation of all organs seem to be the future, being the most physiological way to protect organ function. However, use of ECMO for in vivo normothermic preservation, or use of autologous oxygenated blood for ex vivo preservation require a large logistic organization. When adding oxygenation and circulation devices, this procedure requires a specialized technician to monitor pressure and perfusate flow resistance, almost as for cardiac surgery.

Infections

Bacteriological samples (blood, urine, bronchial samples) are always performed during the resuscitation of the potential donor, but the results will not be known most often after the removal of organs. Severe bacterial or mycotic sepsis (septicemia, peritonitis, meningitis infections) contraindicates organ removal. A culture of preservation fluid of the organ removed is systematic. Despite cold storage, which decreases bacterial proliferation, graft and recipient infection had already been described. We can suppose that with normothermic perfusion or conservation, the infectious risk could increase. In the field of heart and lung surgery, infection of ECMO circuits was described. In a case control study, Pieri [29] found 48% of patients treated with ECMO had infections. Gram-negative bacteria were the predominant pathogens, and Candida albicans was the most frequent isolated microorganism. These data were confirmed by Pluim [30] who retrospectively studied fungal ECMO infections, which reach 5%, increasing patient mortality.

Conclusion

Promising experimental and clinical results in the field of normothermic perfusion make challenge cold preservation. The most suitable and physiological method seems to be a normothermic perfusion and conservation with autologous oxygenated blood using Extra Corporeal Membrane
Oxygenation or Regional Normothermic Circulation. The major limitations are an increased logistics means and until nowadays the impossibility for normothermic organ transportation. With normothermic conditions, the risk for infection of perfusion circuits and organs remains unknown and could be a serious concern.

Disclosure of interest

The authors have no conflicts of interest to declare in relation to this review.

References