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Renal warm ischemia in organ donors after circulatory death

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Renal warm ischemia in organ donors after circulatory death

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Renal warm ischemia in organ donors after circulatory death

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Title Page

Title

Renal warm ischemia in organ donors after circulatory death

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Abstract

Chronic kidney disease is the most common type of organ failure worldwide, with a prevalence of 13.4% for all stages. Organ transplantation is the only curative option for end-stage kidney failure. However, shortage in organ donors remains a major obstacle in organ transplantation, with donation after circulatory death being the most viable path to increase the donor pool. The circumstances that surround this type of donation are different from donation after brain death, namely concerning warm ischemia times, which are longer and may preclude a successful transplantation. This article describes the pathophysiology of warm ischemia and summarizes recent developments in technological and methodological practices that mitigate the mechanisms of warm ischemia. Anoxia, mitochondrial dysfunction, calcium overload, oxidative and nitrosative stress, immune response and no-reflow are the main mechanisms by which ischemia leads to cell death and organ dysfunction. In-situ oxygenated recirculation, abdominal normothermic organ recirculation, abdominal hypothermic organ recirculation and ex-vivo machine perfusion ensure continued organ perfusion and prevent prolonged warm ischemia in organ donation. These, coupled with the optimization in the identification and assessment of potential donors after circulatory death may lead to a significant increase in the number and success rates of organ transplantation worldwide.

Keywords

renal transplantation; organ transplantation; organ donation; warm ischemia; donation after circulatory death; cellular mechanisms; ECMO; controlled DCD; uncontrolled DCD

Abbreviations

20-HETE	20-hydroxyeicosatetranoic acid
AHOR	Abdominal hypothermic oxygenated recirculation
ANOR	Abdominal normothermic oxygenated recirculation
CAMK	Calmodulin-dependent protein kinase
DBD	Donors after brain death
DCD	Donors after circulatory death
DGF	Delayed graft function
ECMO	Extra-corporeal membrane oxygenation
IABP	Intra-aortic balloon pump
IR	Ischemia-reperfusion
ISP	In-situ perfusion
MP	Machine Perfusion
MPT	Mitochondrial permeability transition
MPTP	Mitochondrial permeability transition pore
NOX	NADPH oxidase
NRP	Normothermic regional perfusion
PAF	Platelet activating factor
PNF	Primary non-function

RNOS	Reactive nitrogen oxide species
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCS	Static cold storage
UNOS	United Network for Organ Sharing
WLST	Withdrawal of life-sustaining treatment
XO	Xanthine oxidoreductase

Introduction

Chronic kidney disease is the most common type of organ failure worldwide, with a prevalence of 13.4% for all stages of chronic kidney disease and 0.1% for end-stage disease. It represents a global health burden with a high economic cost to health care systems worldwide: according to a Swedish study, among chronic kidney disease patients stage 4 or 5, those subjected to hemodialysis have the highest annual cost (86000€) while transplanted patients have the lowest (15500€)[1]. Chronic kidney disease is also an independent risk factor for cardiovascular disease: all stages are associated with increased risks of cardiovascular morbidity, mortality and decreased quality of life[2].

Organ transplantation is currently the only curative option for end-stage kidney failure, with lower cardiovascular mortality and event rates and least economic burden when compared to all forms of dialysis[3]. However, the gap between the number of worldwide performed transplants and the number of patients on a waiting list is still considerable: as of 2016 only 62333 of the 279910 patients (22.27%) on waiting list were subject to a kidney transplant[4]. This leaves an enormous portion of patients dependent on dialysis, some of which will die due to complications of the disease, inefficiency of the treatment of cardiovascular disease, which is the major cause of death in patients with end-stage kidney disease.

Increasing the number of donors after circulatory death (DCD) is the most viable way of expanding the organ donor pool[5]. Many countries have already made legal and social efforts to include DCD, either under controlled, uncontrolled or both settings, with very positive results: in the Netherlands, from 2000 to 2009, DCD contributed from 22% to 50% of all deceased organ donors; in the United Kingdom, over the same period, DCD composed 34% of all deceased donors. Utilization rates in these countries consisted of 90% and 93%, respectively[6].

With such a fast inclusion of DCD across diverse regions, attention must be drawn into understanding the particular conditions of death and organ ischemia surrounding these donors and their effect on organ function. Such circumstances are very different from those in donors after brain death (DBD) and therefore the approach to the two must be distinct to increase the odds of a successful transplantation and decreased long term complications for the organ recipients. To achieve this goal, clinicians must be familiar with the cellular and subcellular mechanisms of ischemia, their interaction and their overall contribution to the observed kidney injury.

Considering the high potential of injury in kidneys from DCD due to increased warm and cold ischemic times, preventive approaches must be undertaken to mitigate ischemic injury, tackling the ischemia enhancing mechanisms either individually or as a group, through mechanical, pharmacological and genetic approaches.

In this article, an insight into the cellular and subcellular mechanisms of warm ischemic injury in kidney transplants from DCD is presented. Special focus will be devoted to the existing preventive and mitigating approaches, mainly the already clinically trialed interventions.

Methods

Eligible studies and articles were identified by an electronic search of PubMed and Scopus, involving studies published from 1965 and 2019. The sensitive search strategy combined the following keywords: *warm ischemia; ischemic injury; kidney injury; reperfusion injury; hypoxia; kidney transplant*; non heart beating; donation after cardiac death; donation after circulatory death; donation after brain death; and organ preservation*. All articles and cross-referenced studies from retrieved articles were screened for pertinent information and reviewed by all authors.

Inclusion criteria consisted in experimental and review articles, systematic or not, published as original studies with available abstracts. Publications not written in English were excluded.

Results

Specific conditions in donation after circulatory death

Although in the inceptive development of organ transplantation the first donors were DCD, donation after brain death (DBD) has been, since the Harvard Medical School pivotal report defining brain death[7], the predominant form of donation in many countries[4]. This can be explained by the fact that medical management of the brain-dead donor ensures organ viability for a far longer period of time and in a much more uneventful manner than in the settings of DCD (mainly uncontrolled DCD). In other words, although a timeline exists, there is no immediate danger to organ viability in DBD. Between the diagnosis of brain death and organ retrieval, consent can be obtained from the family (if presumed consent is absent), all concerned entities can be notified and organs are retrieved in a variable period of time after the diagnosis.

Despite DBD having more ideal conditions in regards to organ ischemia time and overall feasibility of the procurement procedures, both patient and graft survival and mid and long term outcomes are similar in kidneys transplanted from DBD and DCD[8]. Moreover, despite higher rates of delayed graft function (DGF) and acute rejection in DCD donor kidney transplants, subsequent outcomes in DCD donor kidney transplants with DGF are better than in DBD donor kidney transplants experiencing DGF, and similar to outcomes in DCD donor kidney transplants without DGF[9]. Primary non-function (PNF) is extremely low in DCD, even within extended-criteria donors[10]. An exaggerated focus on short term outcomes, namely DGF, leads to the potential neglect of this group of donors.

In the context of DCD, overall ischemia times are longer yet variable. Nonetheless, in uncontrolled DCD (donors after circulatory death Maastricht Classification categories I and II

[11], *Table 1*), resuscitation efforts ensure a low-flow environment which prevents full on ischemia, although with intermittent periods of no-flow. In spite of this, uncontrolled DCD often spend a significant amount of time in no-flow or low-flow environments until organ retrieval occurs or the donor is cannulated for extracorporeal circulation – in one Portuguese center, a maximum of 150 minutes is allowed from cardiac arrest to organ preservation, in this case through abdominal normothermic oxygenated recirculation (ANOR) [12]. In these settings, this period of time corresponds to the warm ischemia time.

In regard to controlled DCD (donors after circulatory death Maastricht Classification categories III and IV [11]), duration of warm ischemia is usually shorter, yet variable. In these settings, the sum of the agonal phase (defined as the time from withdrawal of life-sustaining treatment to cardiac arrest), the ten minutes no-touch period and the time to organ preservation is equivalent to warm ischemia time. The equivalence between warm ischemia time and the sum of these periods is debatable because the agonal phase comprises an initial time of normal cardiovascular and respiratory function that ensures normal blood flow and organ oxygenation. In all Netherlands' institutions, maximum agonal phase duration according to the organ retrieved are as follows: <60 minutes for liver, lung and pancreas donation and <120 minutes for kidney donation[13].

Cellular mechanisms of warm ischemic injury

Metabolic adaptation to anoxia

The onset of hypoxia as a result of the decrease of blood flow is the main driver of extracellular, cellular and subcellular changes that occur during warm ischemia. Within the first few minutes of ischemia, absence of oxygen and changes in cellular oxeredox states induce

the activation of anaerobic glycolytic metabolic pathways as alternative ATP sources. However, this results in the production of only a fraction of the ATP produced under aerobic conditions, which is insufficient to meet cellular demands[14].

Concomitantly with the activation of glycolytic pathways and the exhaustion of substrate reservoirs, toxic metabolic products start to accumulate, namely inorganic phosphate, protons, creatine, glycolysis products, H^+ , lactates and NADH. The accumulation of these products cause both toxic and osmolar damage to the cells, eventually resulting in cell death[15].

Ultimately, the glycolytic pathway is fully inhibited by the sum of mainly three factors: (1) accumulation of H^+ , lactates and NADH that inhibits glyceraldehyde phosphate dehydrogenase, thus stopping the glycolytic pathway; (2) exhaustion of cellular glycogen reservoirs; and, most importantly, (3) ATP exhaustion that prevents the phosphorylation of fructose-6-phosphate[15]. This, coupled with the inhibition of other energy substrates and pathways, such as phosphocreatine, leads to the full arrest of ATP production.

Although variable in the human being, attempts have been made to clarify the amount of time of warm ischemia that results in irreversible damage to the tissue. In a rabbit kidney model, should the ischemia time last longer than 24 hours, ATP synthase activity is irreversibly lost and definite cell death ensues[16].

Calcium overload

Mitochondrial dysfunction is one of the main drivers of cell death, either by apoptosis or necrosis, especially during reperfusion injury. A great contributor to this dysfunction is calcium accumulation within the cell and within the mitochondria itself, specially upon reperfusion, where restoration of circulation delivers calcium to the cell, in a rapid and sudden fashion.

Calcium overload occurs mainly by three different pathways (Figure 1):

1. During ischemia and due to ATP exhaustion, Na/K-ATPases stop pumping Na^+ out of the cell. This accumulation of Na^+ would lead to severe changes in membrane

potential which would result in rapid cell death. Therefore, extrusion of Na^+ becomes paramount. One of the transporters used for this purpose is the membrane $\text{Na}^+/\text{Ca}^{2+}$ antiporter, that normally expels Ca^{2+} out of the cell. By reversing its direction, this antiporter removes two atoms of Na^+ in exchange for one atom of Ca^{2+} , leading to intracellular calcium accumulation [17].

2. During ischemia, the activation of the glycolytic pathway leads to the accumulation of lactate, protons and NAD^+ , causing a sudden and severe drop in cellular pH. To counteract this drop, membrane Na^+/H^+ exchanger is activated, leading to an intracellular accumulation of Na^+ . As discussed before, the cell cannot sustain this accumulation of Na^+ and the mechanism of calcium overload is the same as described above[18].
3. Due to the overall metabolic changes within the cell, Ca^{2+} reuptake into the endoplasmic reticulum by the SERCA ATPase is impaired, whereas Ca^{2+} release through the ryanodine receptor is fully enhanced. Although not as substantially important as the aforementioned mechanisms, this also contributes to the intracellular calcium overload observed in warm ischemic injury[19].

In order to maintain mitochondrial membrane potential in face of the calcium overload, transport and retention of calcium within the mitochondria is necessary. This calcium accumulation in the mitochondria causes inhibition of complex I and later of complexes III and IV and, more importantly, activation of the MPT (mitochondrial permeability transition), a nonselective pore in the inner mitochondrial membrane that is activated in certain pathological conditions such as ischemia[20]. MPT response is one of the main drivers of cell death during ischemia and will be discussed below.

Elevation of Ca^{2+} also causes the activation of normally inactive enzymes and proteases, such as CAMK and calpains, that destroy a variety of cell components (cytoskeleton, ER and mitochondrial proteins), therefore contributing to cell death[21].

Further mechanisms of mitochondrial dysfunction

During ischemia, the lack of circulation prevents oxygen delivery to the organs. Because cells exhaust the remaining oxygen rapidly, electron flow through the respiratory chain is inhibited, leading to the arrest of ADP phosphorylation through the ATP synthase. In an attempt to maintain membrane potential in face of the inhibited electron transfer, ATP synthase actually starts acting in reverse, consuming the almost depleted ATP reservoirs of the cell.

As discussed before, due to calcium overload, among other factors, the MPT response is activated. This is a crucial step in the process by which ischemia leads to cell death. Mitochondrial permeability transition pore (MPTP) is a non-selective pore located in the inner membrane of the mitochondria that allows passage of molecules under 1500 Daltons in molecular weight[22]. Because MPTP is inhibited by the low pH in the ischemic period, MPT response is only activated upon reperfusion, mostly due to rapid increase in Ca^{2+} and in reactive oxygen species (ROS) [23]. The opening of the MPTP allows the passage of H^+ ions into the matrix, which instantly leads to the irreversible loss of the mitochondria membrane potential, resulting in cell death in a rather rapid fashion[24].

Oxidative and Nitrosative Stress

Although timely restoration of blood flow and organ reperfusion is the only way of ensuring cell survival following an ischemic event, reperfusion itself presents a threat to the cell in what is commonly called the “oxygen paradox”. Despite restoring aerobic ATP production, reentry

of oxygenated blood into the ischemic tissue results in the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive nitrogen oxide species (RNOS). Among other things, these molecules are responsible for damaging cell components, inducing cell death, stimulating the production of pro-inflammatory mediators and facilitating leukocyte cell adhesive interactions[25].

The traditional view of oxidative cell damage, first described in 1985[26], defined it as an imbalance between pro- versus antioxidant compounds favoring the formation of prooxidant molecules that would directly lead to cell damage. Recently, three targets have been identified in oxidative stress: (1) direct molecule damage of all cell components (DNA, protein, lipids and carbohydrates), (2) irreversible modification of key cell regulatory components through covalent, oxidative and nitrosative changes[27] and (3) the formation of nonradical oxidants such as H_2O_2 , that in turn mediate cell dysfunction through several mechanisms[28].

Oxidative Stress – ROS and RNOS

As discussed above, ROS are one of the major players in oxidative damage in ischemic reperfusion injury. Within this category, superoxide anion radical (O_2^-) is the originally produced ROS and gives rise to all the other ROS and RNOS that participate in oxidative stress injury.

Superoxide is mainly formed by enzymatic sources: XO (xanthine oxidoreductase), NOX (NADPH oxidase), cytochrome P450 oxidases and NOS (nitric oxide synthase). The main source of superoxide varies across species, tissues and even individuals. In kidney tissue the mechanisms of oxidative damage in ischemia and reperfusion injury appear to be very similar to those found in other human tissues, with particular emphasis on the post-ischemic oxidation and release of PAF and other lipids [29, 30].

- 1) XO (xanthine oxidoreductase): in the kidney, endothelial cells are particularly rich in XO, which requires hypoxanthine and oxygen to fuel the production of

superoxide. During ischemia, due to ATP depletion, and in reperfusion, due to the oxygen cell inflow, both hypoxanthine and oxygen suffer a sudden increase, leading to a burst in the production of superoxide. Inhibition of XO has been shown to reduce Ca^{2+} overload and markers of oxidant stress, showing the importance of this enzyme in the production of superoxide[31].

- 2) NOX (NADPH oxidase): two types of this enzyme have been shown to be involved in damaging superoxide production in the context of ischemia reperfusion injury. The first one is the NOX present in phagocytic leukocytes responsible for the production of oxidative compounds that ensure a suitable host defense[32]. In this scenario, superoxide is rapidly dismutated to hydrogen peroxide which in turn originates hypochlorous acid. Lastly, NOX is also present in vascular endothelial cells, although in a much lower concentration than in leukocytes. Whatever subtle effects endothelial NOX may have under normal conditions, in ischemia and mainly during reperfusion, both these types of NOX contribute to the production of superoxide in a sufficient amount to cause oxidative stress cellular damage[33].
- 3) Cytochrome P450 enzymes: these enzymes are responsible for the univalent oxidation and reduction of xenobiotic compounds as well as other cellular molecules. The majority of these enzymes are present in the liver, although a small amount can also be found in endothelial cells, including in the kidney. Although the precise role and importance of cytochrome P450 enzymes in ischemic injury is still unclear, they appear to contribute to cellular damage through the generation of ROS, dihydroxydecanoic acid and, most importantly, 20-hydroxyeicosatetranoic acid (20-HETE). 20-HETE is a potent vasoconstrictor with a yet unclear role in acute kidney injury, as its inhibition protects against damage in unilateral ischemic damage but enhances it in bilateral kidney injury [34-36].

- 4) Respiratory chain: Over 90% of the oxygen that enters the cell is reduced to water by the respiratory chain located inside the mitochondria, during the process of ATP production. However, under normal conditions, 1-2% of the oxygen is reduced to superoxide in Complex I and Complex III enzymes, mainly due to “electron leak”, which is increased during reperfusion. This, coupled with the exhaustion of the cellular antioxidant capacities, inevitably leads to severe oxidative damage through an imbalance in pro- and antioxidant sources[37].

The effects of the produced ROS and RNOS are varied and almost all contribute to cell damage and death, especially during the reperfusion phase of the ischemia reperfusion injury. As discussed, superoxide is the primary oxidant and all other RNOS eventually derive from it. We can separate superoxide effects into direct and indirect.

In regards to direct effects, superoxide can directly oxidize several enzymes such as aconitase, fumarase, NADH dehydrogenase and creatine kinase[25]. The inactivation of these enzymes would have catastrophic consequences to cell function. However, superoxide has a very small lifetime as is, because of rapid spontaneous and catalytic (by superoxide dismutase) conversion to hydrogen peroxide (H_2O_2), which prevents severe cell damage.

Indirect effects of superoxide arise from the products originated by its conversion. Hydroperoxyl radical ($HOO^\bullet$) is the conjugate acid of superoxide and is originated through spontaneous conversion. Its production increases in low pH environments, such as in ischemia. Hydrogen peroxide, although the least reactive of all ROS, can directly act as a second messenger and modulate cell signaling. However, it can also give rise to the highly oxidative compound hydroxyl ($^\bullet OH$) and can directly damage cell components that contain heme proteins. Lastly, superoxide can originate the highly toxic peroxynitrous acid ($ONOOH$), through a reaction with NO and further protonation. All these compounds have the potential to damage all cell components through irreversible oxidation.

Immune response in ischemia

It is now well established, not only in the context of DCD but in all ischemic conditions, whether transient or permanent, that the activation of the immune response contributes to further cell damage. However, this is still a matter of great debate, as conflicting reports of protective and damaging roles of the immune major players have been presented[38]. In general, deposition of natural antibodies, complement activation and neutrophil infiltration have been identified as the initiators of immune response in ischemia. Neutrophil and T cell infiltration are the two most important cell types in IR injury and are described below.

Neutrophil infiltration

Neutrophil infiltration in an ischemic/reperfusion episode occurs as early as 30min after reperfusion and it can be seen both in animal subjects and patient biopsies[39]. This happens due to a highly effective chemotactic gradient initiated by the release of several inflammatory messengers (TNF- α , IL-6, monocyte chemotactic protein 1, RANTES, macrophage inflammatory protein 2, among others) by the dendritic cells resident in the kidneys[40, 41]. This chemotactic gradient allows fast migration of neutrophils into the reperfused tissue, resulting in increased vascular permeability and cell damage. Of particular relevance are the molecules ICAM-1, P-selectin and IL-8, which potentiate neutrophil adhesion and infiltration (crucial for tissue damage) in a continuous fashion, maintaining a constant inflow of immune cells to the newly reperfused tissue[42].

Upon arrival, neutrophils produce a large amount of ROS in the outer medulla (through its intracellular NADPH oxidase, which is activated when the neutrophil adhesion takes place), proteinases, myeloperoxidase and cationic peptides[41, 43]. The large amount of neutrophil affluence to the ischemic tissue, along with platelet and red blood cells, cause microvascular

dysfunction upon reperfusion, leading to further tissue necrosis and exacerbated immune response, thereby contributing to the no-reflow phenomenon[39, 44].

T cells

The exact role of T cells and their direct effects on renal cells in the IR injury is still most unclear, though recent animal and human models have shed a light on the detrimental action of these cells, which exacerbate inflammatory injury in an acute ischemic event. Numerous experiments in animal models have proven that inhibition of T cell function, either by blocking the stimulation process or by direct cell depletion, is beneficial in the context of an ischemic insult and mitigates cell damage and death [45-48]. More specifically, after an ischemic event, transient T cell depletion of CD4+ T cells promoted renal protection, increased animal survival and was associated with less acute tubular injury and earlier regeneration[49].

IR injury caused by T cells takes place not only after the release of alloantigens by the dying ischemic tissue (as previously thought), but also during the first critical phase after the cessation of blood flow. In this stage, activated T cells adhere to the endothelium of the capillary network, slow down the already diminished circulation and re-enter the systemic circulation shortly after, a phenomenon known as “hit-and-run”. Expression of ICAM-1, an adhesion molecule that facilitates leukocyte adhesion and infiltration, increases very shortly after an ischemic event, especially when compared to acute toxic injury[50]. As a result, the use of agents that prevent cell adhesion by blocking ICAM-1 mitigate IR damage, particularly in combination with lymphopenia inducing drugs[51]. Indeed, endothelial cells are the main drivers of T cell activation in this context, having the ability to provide costimulatory signals to circulating cells (CD40, CD80 and CD86) and act as antigen presenting cells[52]. This concept challenges the classical view of T cell activation, taking place in a sterile environment

and in the absence of an antigen stimulus, which has already been proven in a kidney model[45].

No-reflow phenomenon

The no-reflow phenomenon in renal ischemia, first described in 1971[53], happens immediately after the reestablishment of blood flow in an ischemic event and consists of the failure of a large number of capillaries being reperfused. This phenomenon happens not only in the kidney but also in other major organs (brain, heart, small intestine and skeletal muscle).

The initial hypothesis to explain this phenomenon proposed microvascular thrombosis as the pathological mechanism behind the capillary obstruction. However, microvascular thrombus formation is rarely observed and heparin treatment is not effective in restoring capillary perfusion after an ischemic event in skeletal muscle[54].

Several indirect evidence has put leukocyte infiltration, namely neutrophils, at the center stage of the development of this phenomenon. It has been proven that there is a strong direct correlation between the extent of the neutrophil infiltration in the ischemic tissues and the area of capillaries that fail to be reperfused, which was corroborated by the observation that no-reflow is almost totally abolished by neutrophil depletion in the heart brain and skeletal muscle[54].

Leukocyte infiltration brings about capillary no-reflow phenomenon mainly by three mechanisms: leukocyte impaction in the capillaries, formation of edema and the production of oxidant species.

1. The notion that leukocyte impaction contributes to this phenomenon comes from the fact that these are stiff and large cells that must suffer significant deformation in

order to enter and travel through the small capillary networks. Given low pressure that drives blood through the capillaries and the low pH during ischemia (which increases the stiffness of leukocytes), there is a higher probability of leukocyte impaction that blocks reperfusion upon the restoration of circulation[54].

2. Formation of edema under ischemic conditions results from the neutrophil dependent microvascular endothelial barrier disruption. As a result, fluid and proteins leave the vessels according to its gradient and accumulate in the tissues causing edema. It has been proven that this formation of edema secondary to neutrophil dependent increase in vascular permeability contributes to the genesis of no-reflow in a skeletal muscle model. Formation of edema was associated with a marked decrease in the number of patent capillaries and treatment with phalloidin and hyperosmotic saline-dextran solution (that alter the gradient between the blood and the tissues and impede fluid flow to the interstitial space) prevented edema formation and attenuated the reduction in the number of patent capillaries[55].
3. However, changes in the external pressure are not the only cause of vessel closure. Instead, active vasomotility also plays an important role in the development of no-reflow, through the release of oxidant species by infiltrating leukocytes, which have a powerful vasoconstrictor effect, as discussed previously. It has been proven in an animal model that treatment with L-arginine alone or in combination with antioxidative vitamins prior and during a limb ischemic episode reduces interstitial edema by 31% and 40%, respectively, prevented microvascular constriction and preserved blood flow after reperfusion without development of no-reflow phenomenon[56].

Preventive and therapeutic approaches to warm ischemic damage

Although the general mechanisms of cell ischemic damage have been extensively studied, donor pretreatment to prevent and mitigate warm ischemic injury is still under investigation and clinically trialed interventions to achieve this goal are scarce, especially in the context of donation after circulatory death. Timely identification of potential DCD, accurate assessment of the probability of transplantation success for each donor, in-situ oxygenated recirculation under normothermic or hypothermic conditions, ex-vivo machine perfusion and cold storage with different solutions are the so far implemented methods of improving utilization rates.

Identification and assessment of potential donors after circulatory death

As discussed previously, the implementation of DCD programs is the most effective way to increase organ donation and transplantation[5]. In these programs, and because of the special conditions that involve these types of donor, criteria for the identification and validation of the donors must be accurate and under constant review so as to identify potential pitfalls and improve identification and utilization rates.

In the context of controlled DCD (donors after circulatory death Maastricht Classification categories III and IV [11]), the time between withdrawal of life-sustaining treatment (WLST) and cardiac arrest is one of the most important parameters to assess the suitability of the donor's organs for transplantation and is related to the degree of hemodynamic and respiratory support and the patients' respiratory, neurologic and circulatory condition[57]. Several studies have attempted to identify possible variables that predict a rapid death after WLST (maximum 2h after WLST, depending on the organ). Controlled mechanical ventilation, norepinephrine administration, absence of brain reflexes, neurologic deficit, and absence of cardiovascular

comorbidities were identified as independent risk factors for cardiac death under 60 minutes after WLST in a prospective study in the Netherlands[13]. The United Network for Organ Sharing (UNOS) critical pathway for donation after cardiac death suggests ventilatory support for respiratory insufficiency, mechanical circulatory support, severe disruption in oxygenation, pharmacologic circulatory assist, intra-aortic balloon pump (IABP) and inotropic support as indicators of probable death under 60 minutes after WLST[58]. All these criteria can be used to accurately identify and assess the probability of a successful organ retrieval and transplantation. However, a solid and validated scoring instrument for the objective assessment of potential DCD donors is still inexistent and should be a topic of research in this area.

In-situ Oxygenated Recirculation

In DCD (especially in an uncontrolled setting) warm ischemia times are far higher than in DBD and, as such, there is a much higher probability of IR injury to the graft. It is therefore important to somehow maintain oxygenation of the organs between patients' death (with consequent cessation of circulation) and organ retrieval. With the growing implementation of DCD programs to expand the donor pool, attention has been drawn to the development and study of new organ preservation methods in these settings, namely hypothermic and normothermic regional perfusion.

In the context of DCD, there is normally a no-touch period with varying duration between different countries (extending from 5 to 15 minutes). After this, vessel cannulation of the vessels is performed and extracorporeal circulation is initiated. Again, there is an enormous variation in inclusion criteria and in techniques used in different centers: surgical vs percutaneous cannulation, extracorporeal membrane oxygenation (ECMO) vs standard bypass, continuous vs pulsatile circulation, occlusion of the aorta vs non-occlusive methods, etc. These

regional and national differences in protocols, whether from local preferences in techniques or different legal frameworks, prevent a completely objective comparison in terms of outcomes.

Abdominal Hypothermic Oxygenated Recirculation (AHOR)

Hypothermic regional perfusion was developed to meet the potential benefits of organ maintenance under hypothermic conditions: more efficient cooling, reduced warm ischemia and continuous gas exchange. The technique involves cooling the perfusate (diluted blood solution) to a temperature ranging from 4 to 20 °C and maintaining recirculation from the end of the no-touch period to surgical organ retrieval. Despite continuous gas supply to the reperfused organs, after 20 minutes of hypothermic recirculation, oxygen consumption is minimal, owing to the decrease in metabolic processes caused by the subnormothermic temperatures[59]. In comparison to abdominal normothermic oxygenated recirculation (ANOR), this is one of the major potential advantages of AHOR.

The clinical results of this technique vary considerably between different reports: initial graft function ranges from 9%-35%, delayed graft function (DGF) from 21%-85% and primary non-function (PNF) from 4%-6% [60-63].

Abdominal Normothermic Oxygenated Recirculation (ANOR)

Normothermic regional perfusion (NRP) has the undeniable advantage of maintaining normal cell metabolism without the deleterious effects of ischemia. To the organ itself it is as if the donor's heart is still beating and consequently all the aforementioned mechanisms of cell injury are stopped at its start. Cell integrity is preserved and it is expected that overall organ function follows suit.

The efficacy of NRP in comparison with SCS and in situ perfusion (ISP) in DCD has been established, both in short term outcomes such as PNF and DGF and overall graft survival at 1 year post transplantation [64-66], placing NRP as the undoubtedly preferred method of organ preservation and donor conditioning in DCD. Despite this, high quality evidence comparing the short and long term efficacy of AHOR and ANOR still lacks and further research in this area is warranted.

Ex-vivo Machine Perfusion

Organ preservation after the cessation of circulation and organ retrieval has been an object of intense study since the middle 19th century, when the first attempt to perfuse an isolated organ was made[67]. The concept of organ perfusion after death was successively redefined and improved with the usage of small pumps and whole blood as the perfusate[68, 69], until 1967, when a combination of continuous perfusion and hypothermic organ storage was designed and allowed 72-hour preservation of canine kidneys[70]. However, several studies failed to prove any benefit from machine perfusion (MP) when compared to static cold storage (SCS) [71] and since the beginning of the 80's, most recovered kidneys have been preserved through SCS alone, with improving success rates owing mostly to enhanced preservation solutions and recipient immunosuppressive therapy.

Nowadays, most transplanted kidneys retrieved from both DCD and DBD are maintained under SCS, mostly due to the much easier handling and preparation of the organs. Despite this, the use of MP (especially in the context of DCD, where ischemic damage is a far greater concern than in DBD) is increasing, owing both to the solid evidence of the superiority of MP when compared to SCS and to the increasing number of DCD and its importance to the overall transplantation paradigm. A recent Cochrane systematic review concluded that in comparison

with SCS, hypothermic machine perfusion reduces the rate of DGF in kidneys obtained from deceased donors. Moreover, it results in increased survival of the transplanted kidney and overall costs saving [72]. Notwithstanding, insufficient data prevents the study of normothermic machine perfusion and its comparison to hypothermic machine perfusion.

Future perspectives in organ preservation

Organ transplantation is still a developing area of medicine and research in this field is of crucial importance. Although the mechanisms of ischemia are now understood, little to no direct therapy aimed at these specific mechanisms is well studied and being employed at the moment. Tackling these mechanisms, improving donor detection and organ preservation methods are amongst the most important milestones to be achieved in organ transplantation.

Control of coagulation was one of the first proposed methods to decrease IR injury. Theoretically, heparin administration would prevent macro and microvascular thrombosis, which would mitigate the no-reflow phenomenon. However, it has been shown that little to no microvascular thrombosis occurs in this context [54] and the effect of heparin administration on graft function is still under intense discussion in the transplantation community[73]. Moreover, the pre-mortem administration of heparin is ethically questionable, since it has no benefit and could hasten the death of the patient[74].

Ischemic preconditioning, though feasible and effective in living donor transplantation and DBD[75], faces the same ethical issues as the administration of heparin, where intervention aimed at improving transplantation outcome in the not-yet-dead donor is troublesome from a moral point of view.

Conclusion

Overall ischemia time is still the major determinant of organ viability in the context of organ donation from deceased donors. Understanding the pathophysiology of warm ischemia, its differences from cold ischemia and finding ways to decrease its time to the least possible value, along with donor conditioning that mitigates warm ischemia mechanisms, optimizes organ viability. This is particularly important in DCD, where ischemia times are unpredictable and may preclude organ transplantation.

Coupled with legal and social changes that allow all forms of organ donation (DBD, controlled and uncontrolled DCD) and proper donor identification and assessment for organ transplantation, this understanding and mitigation of warm ischemia can greatly increase the number and success rates of kidney transplantation.

Keeping the status quo on this matter will result in a large proportion of patients on the organ transplant waiting list never being transplanted. Efforts must therefore be made, through scientific research, medical training and policy changes in order to maximize the number and the success of kidney transplantations, which is the only hope for a tremendous number of end-stage kidney disease patients worldwide.

Appendix

Figure 1

Legend

Calcium overload mechanism – 1) Due to ATP exhaustion, Na/K-ATPases stop pumping Na^+ out of the cell. There is an accumulation of Na^+ and the extrusion of Na^+ becomes paramount. One of the transporters used for this purpose is the membrane $\text{Na}^+/\text{Ca}^{2+}$ antiporter, that normally expels Ca^{2+} out of the cell. By reversing its direction, this antiporter removes two atoms of Na^+ in exchange for one atom of Ca^{2+} , leading to intracellular calcium accumulation.

2) The activation of the glycolytic pathway leads to the accumulation of lactate, protons and NAD^+ , causing a sudden and severe drop in cellular pH. To counteract this drop, membrane Na^+/H^+ exchanger is activated, leading to an intracellular accumulation of Na^+ . The cell cannot sustain this accumulation of Na^+ and the mechanism of calcium overload is the same as described above

3) Due to the overall metabolic changes within the cell, Ca^{2+} reuptake into the endoplasmic reticulum by the SERCA ATPase is impaired, whereas Ca^{2+} release through the ryanodine receptor is fully enhanced. Although not as substantially important as the aforementioned mechanisms, this also contributes to the intracellular calcium overload observed in warm ischemic injury.

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Annexes

Table 1

Category I	Found dead	Sudden unexpected cardiac arrest
Uncontrolled	IA. Out-of-hospital (OH) IB. In-hospital (IH)	(CA) without attempts of ressuscitation by a medical team
Category II	Witnessed cardiac arrest	Sudden unexpected irreversible CA
Uncontrolled	IIA. OH IIB. IH	with unsuccessful resuscitation by a medical team
Category III	Withdrawal of life sustaining therapies	Planned withdrawal of life sustaining therapies – expected CA
Category IV	CA while brain-dead	Sudden CA after brain death
Uncontrolled		diagnosis during donor life-
Controlled		management but prior to planed organ recovery

Table 1- Modified Maastricht Classification of DCD

Figure 1

Calcium overload mechanism – 1) Due to ATP exhaustion, Na/K-ATPases stop pumping Na^+ out of the cell. There is an accumulation of Na^+ and the extrusion of Na^+ becomes paramount. One of the transporters used for this purpose is the membrane $\text{Na}^+/\text{Ca}^{2+}$ antiporter, that normally expels Ca^{2+} out of the cell. By reversing its direction, this antiporter removes two atoms of Na^+ in exchange for one atom of Ca^{2+} , leading to intracellular calcium accumulation. 2) The activation of the glycolytic pathway leads to the accumulation of lactate, protons and NAD^+ , causing a sudden and severe drop in cellular pH. To counteract this drop, membrane Na^+/H^+ exchanger is activated, leading to an intracellular accumulation of Na^+ . The cell cannot sustain this accumulation of Na^+ and the mechanism of calcium overload is the same as described above 3) Due to the overall metabolic changes within the cell, Ca^{2+} reuptake into the endoplasmic reticulum by the SERCA ATPase is impaired, whereas Ca^{2+} release through the ryanodine receptor is fully enhanced. Although not as substantially important as the aforementioned mechanisms, this also contributes to the intracellular calcium overload observed in warm ischemic injury.

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TABLES

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