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CURRENT STRATEGIES TO PREVENT ISCHEMIA REPERFUSION INJURY IN ORGAN TRANSPLANTATION

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ABSTRACT

Solid organ transplantation is inherently associated to ischemia reperfusion injury (IRI) since the operative procedure always implies the harvesting, preservation and implantation of the organ, which result in several hours of ischemia followed by reperfusion. Several strategies have been proposed to mitigate IRI in organ transplantation. However, most of them are in experimental phases and currently few are implemented in the clinical field. These strategies can be applied in 3 different stages of the transplantation procedure: on the organ donor, known as donor pre-treatment; during preservation, when ischemic organ is waiting to be implanted in the recipient or on the transplant recipient. In the present review, we will discuss the different approaches to control IRI damage in solid organ transplantation and the rationale behind them.

Keywords: solid organ transplantation; ischemia; reperfusion; prevention.

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Introduction

Ischemia reperfusion injury (IRI) is established in the tissue after reintroduction of oxygenation and normal irrigation following several minutes of ischemia. This situation can take place in diverse scenarios, such as thrombosis, vascular occlusion or operative trauma, causing different degrees of tissue damage contributing to the physiopathology of these conditions. Solid organ transplantation (TX) is inherently associated to ischemia reperfusion injury since the operative procedure always implies the harvesting, preservation and implantation of the organ, which result in several hours of ischemia and subsequent reperfusion. The degree of IRI has critical influence on initial graft performance; furthermore, higher incidence of acute and chronic rejection has been associated to severe IRI in different TXs. In consequence, minimizing IRI has been a goal in the organ transplantation field [1]. Due to the importance of this entity in different clinical settings, the mechanisms of its production and therapeutic approaches to prevent it have been extensively addressed. There are several excellent revisions that cover the application of different strategies to modulate IRI in non-TX situations [2, 3]. In the present review, we will focus on the strategies to control IRI damage in solid organ transplantation and the rationale behind them.

Mechanisms that mediate IRI in solid organ transplantation

IRI is a dynamic process involving two distinctive but interrelated phases: ischemic organ damage and inflammation-mediated reperfusion injury. Tissue/organ damage is the result of multiple cellular and molecular pathways that we will briefly discuss in the present section. Many of these factors have been targeted by the different therapeutic strategies used in the clinic so far:

- *Hypoxia*: During ischemia, the organ that is deprived of oxygen consumes and depletes the adenosine triphosphate (ATP) pool and other immediate sources of chemical energy. The decrease in ATP production during ischemia leads to accumulation of different metabolic intermediates, such as hypoxanthine, which is the substrate of the enzyme xanthine dehydrogenase/oxidase. Under hypoxic conditions, this enzyme is fully converted into the oxidase form, which will have consequences in later steps after reoxygenation. In addition, during the ischemic event, transcriptional reprogramming takes place, inducing the expression of genes that are controlled by Hypoxia inducible factor 1 (HIF1a) which impact in proinflammatory gene expression such as IL1b and CXCL8. NFκB pathway is also regulated by redox and hypoxia situations [4]. Under tissue hypoxia conditions several proinflammatory factors, such as TNFα and other cytokines are synthesized [5], contributing to local and systemic inflammatory response.

- *Reoxygenation-induced free radical production*: Although blood supply is essential for tissue survival, restoration of blood flow to the ischemic tissue may paradoxically

exacerbate tissue injury. During the ischemic phase different substrates are accumulated, which, in the presence of oxygen will be metabolized by enzyme systems generating reactive oxygen species (ROS). Among them the xanthine oxidase system is the best characterized [6]. The reintroduction of oxygen, which is the limiting reaction during the ischemic phase, triggers the sudden production of free radical species once blood flow is restored. Changes in mitochondrial metabolism at the transition from ischemia to reoxygenation, also contribute to ROS burst [7]. Overproduction of highly reactive oxidant molecular species induces tissue damage and triggers proinflammatory response by direct and indirect pathways. ROS can react in a non-enzymatic manner with virtually all main cell molecules, causing dysfunctions at different critical pathways that may lead to apoptotic, necroptotic or necrotic cell death, depending on the type and the extension of damage. On the other hand, many different molecular signals derived from ROS-induced cellular damage act as damage -associated molecular patterns, triggering the inflammatory response by stimulation of membrane receptors bound to cytoplasmic pathways such as toll like receptors (TLRs), inflammosomes or other damage-responsive proinflammatory pathways [8].

-*Complement system activation*: Animal studies have demonstrated that the complement system plays a pivotal role in mediating inflammation and apoptosis during the development of IRI [9]. Although the molecular basis of the process is not fully elucidated, there is a consensus that changes in the endothelial surface of ischemic organ vessels expose neo-antigens that can react with natural antibodies and activate the complement cascade once blood flow is restored. This event has as a consequence the production of microvascular damage that enhances the deleterious role of the majority of the factors mentioned before [10].

- *Inflammatory events associated with innate response activation*: A hallmark of the reperfusion period is increased leukocyte adhesion to the vascular endothelium [11]. Expression of leukocyte adhesion molecules during the ischemic period allows for their increased anchoring to the vascular endothelium, contributing to the increase in permeability of post -capillary venues and toxic product deposition. Furthermore, enhanced production of proinflammatory chemokines upon reperfusion directs the recruitment of granulocytes to the organ parenchyma. These cells are activated upon contact with the proinflammatory tissue environment and contribute to the tissue damage and exacerbation of the proinflammatory status [12].

- *Brain death induced changes*: Brain death (BD) is the condition that is most widely recognised in the legislation of different countries as criteria for donation. Brain death causes deep changes at physiological, cellular and molecular levels [13]. It has been reported that the quality of organs to be transplanted is impaired in the BD donor [14]. Hormonal and hemodynamic consequences of BD modify perfusion and oxygenation conditions of the organs. Autonomic, metabolic, endocrine and inflammatory cytokine storms occurring during BD contribute to graft injury [15]. BD induced-damage thus exacerbates IRI and has a negative impact on post-transplant outcomes. Furthermore,

organs from living donors (especially kidneys and livers) provide better results compared to those from BD donors [16]. Several studies demonstrated that the combination of BD and IRI potentiates damage, indicating a synergistic effect between BD and IRI [17]. Therefore, treatments that attenuate BD damage also would decrease IRI and improve transplant outcomes.

Strategies to attenuate IRI in organ transplantation

Several strategies have been proposed to mitigate IRI in organ transplantation. However, most of them are in experimental phases and currently few are implemented in the clinical field. These strategies can be applied in 3 different stages of the transplantation procedure (**Figure 1**). The first one is on the organ donor (donor pre-treatment) using preventive strategies when IRI has not yet started. The second is during preservation, when ischemic organ is waiting to be implanted in the recipient. Finally, some treatments can be applied on the transplant recipient.

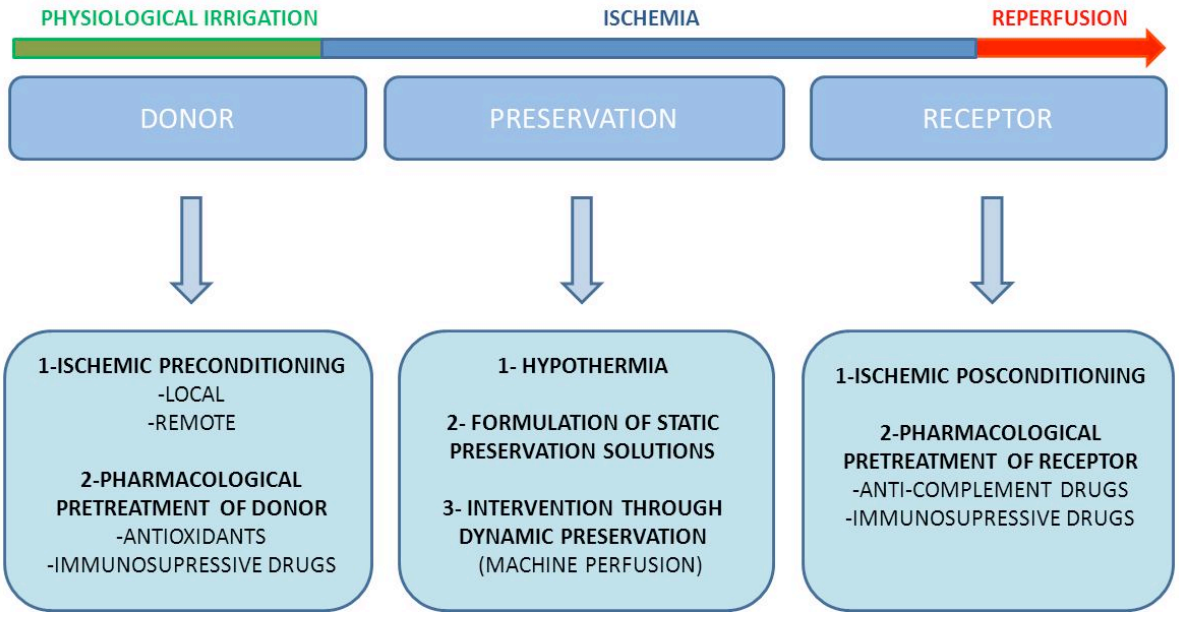

Figura 1: Possible strategies to apply during different stages of the organ transplantation procedure to prevent IRI.

1- Donor pre-treatment:

Donor pre-treatment can be defined as an active treatment to improve organ quality before and after transplantation. Interventions on the donor, aimed to minimize pre-transplantation graft injury, may prevent acute and long-term graft dysfunction. In this section, we discuss the most frequently applied donor pre-treatment in the experimental and clinical organ transplantation field.

1.1- Ischemic preconditioning (IPC): IPC refers to a phenomenon whereby exposure of the target organ to brief periods of ischemia and reperfusion protects it from the deleterious effects of IRI. IPC was first described in 1986 by Murry et al, who showed that this treatment reduced damage in a canine model of myocardial infarction [18]. After this first report, several experimental studies were performed to evaluate the effect of IPC in organ transplantation. They showed that IPC protects many organs against IRI. In liver, kidney, heart, lungs and intestinal transplantation IPC has been shown to reduce the inflammatory response as well as the oxidant stress [19-21].
The mechanism by which IPC results in protection is not fully understood. Several studies have shown that the protective effects are mediated by nitric oxide and adenosine with subsequent activation of signalling networks involving protein kinases and transcription factors such as NFκB [22, 23]. These transcription factors modulate the expression of some genes, resulting in the synthesis of proteins like heat shock proteins (HSP) or superoxide dismutase (SOD) which have been proposed as effectors of IPC protection. Recently, more complex mechanisms have been proposed, including participation of regulatory T cells and endothelial progenitor cells [24, 25].
Little evidence exists regarding clinical application of PCI. In the area of liver transplantation, preconditioned livers showed lower hepatocellular necrosis during the first post-surgical days compared to the untreated group. Despite these positive results, bilirubin levels, neutrophil infiltration and the presence of apoptotic cells were similar in livers who received PCI and controls [26, 27].

1.2- Pharmacological pre-treatment: Different drugs were proposed to treat the donor previous to organ procurement, aiming to prevent IRI. Among them, immunosuppressors and antioxidants are the most used in experimental and clinical studies.
The main role of immunosuppressive drugs in organ transplantation is to prevent or mitigate graft rejection. However, in recent years it has been proposed that these drugs may also attenuate IRI. Using a syngeneic model of kidney transplantation in rats, Cicora et al. demonstrated that tacrolimus (TAC) improves clinical outcomes and reduces necrosis and apoptosis in kidney IRI [28]. TAC immunosuppressive action is due to the inhibition of the nuclear factor of the activated T-cells (NF-AT) and suppression of IL-2 production, which is essential for the clonal expansion of T cells. Although its exact mechanism of action on IRI prevention is not completely elucidated, several studies have shown that TAC may attenuate IRI decreasing ATP depletion, reducing the formation of free radicals, inhibiting calcium-dependent pathways and intracellular signaling pathways such as NFκB [29]. It

has been reported that TAC pretreatment induces arterial relaxation by decreasing calcium, reduces de risk of vasospasm and thrombosis and attenuate IRI in a porcine model [30].
In the area of antioxidant strategies, different drugs aiming to mitigate IRI are being studied. Among these, n-acetylcysteine (NAC) has been positioned in a leading role because of its promising results in several organs and tissues. NAC has a thiol group that is a source of L-cysteine and reduced glutathione, acting as an antioxidant by virtue of its interaction with ROS [31]. In experimental models of intestinal transplantation, NAC confers a protective effect associated with higher levels of nitric oxide (NO) and lowers plasma levels of TNF-α, IL-8 and LDH [32]. In heart transplant performed in rats, NAC significantly improved graft post-transplant function and decreased LDH, TNF-α and IL-1 serum levels. NAC also mitigated myeloperoxidase activity and neutrophil infiltration in the transplanted heart compared with the untreated group [33].

1.3-Brain Death damage attenuation: Most of the scientific works on this topic are focused on renal transplant. In a rat model, Pratschke et al. observed that preconditioning BD donors with steroids reduces IRI in kidney recipient [34]. It has been reported that anti-thymocyte immunoglobulin improves the quality of kidneys obtained from BD donors in an experimental model [35]. In a kidney transplant model, Maio et al. showed that erythropoietin can significantly suppress the renal damage caused by BD [36].

2- Actions applied during the preservation phase:

2.1- Hypothermia: One of the first proven strategies to mitigate IRI and preserve graft integrity was the reduction of cellular activity by decreasing the organ temperature to 2-4 °C. Currently, this strategy is one of the few that have been endorsed by the entire medical-transplantation community and is applied to all organ transplants. It allows, through the amelioration of the IR, to increase the ischemic period before graft is implanted in the recipient [37].
The mechanistic principle behind hypothermia is the decrease in metabolic rate. Although the low temperature delays cell death, hypothermia can not completely prevent ischemic damage. Today, cold storage is achieved using cold preservation solutions, which themselves are formulated to reduce IRI as we will discuss in the following section.

2.2- *Use of different static preservation solutions*: To achieve hypothermia in the graft, two different procedures are performed during the organ procurement. The first involves the vascular infusion with cold liquid, in order to get a quick and uniform cooling of the graft, while providing an appropriate intravascular washing. This process is performed in the donor, just before removing the graft. The second consists of immersing the organ in cold preservation solutions until implanting the graft in the recipient. The composition of these fluids, known as preservation solutions (PS), has varied over the years, by the inclusion of new additives aiming to give organ protection against the effects of IRI.

The cold-static organ preservation for transplantation is based on the use of PS containing specific agents that allow attenuating IRI. A key factor for preservation is the composition of PS, which should have: low viscosity to facilitate washing of intravascular blood, amino acids to improve viability, waterproofing agents and colloids to prevent further edema, and buffers to maintain pH homeostasis.

The solution from the University of Wisconsin (UW) was developed two decades ago and has been extensively used for organ preservation [38]. UW solution includes the trisaccharide raffinose as conserving agent, lactobionate as osmotic waterproofing anion, hydroxyethyl starch as a colloid and phosphate buffer. It also includes the antioxidant glutathione aimed to preserve from ROS damage and allopurinol to inhibit hypoxantine accumulation during ischemic phase [39].

Other solutions used for organ preservation in the clinic are Celsior and Histidine-tryptophan-ketoglutarate (HTK). Celsior is mainly characterized by including mannitol as waterproofing agent, histidine as buffer and the absence of a colloid compound. HTK is a solution which has among its components mannitol, histidine and two amino acids: tryptophan, which functions as membrane stabilizer and antioxidant, and ketoglutarate, a substrate for anaerobic metabolism. It has been reported that HTK solution provides better washing and cooling of the organs that the UW solution. However, these reports are still under study and controversy [40]. Today, various preservation solutions are tested in experimental models. For example, Polysol is a PS with high oncotic pressure and 3 times lesser viscosity than UW. In addition, it is formulated with a polyethylene containing colloid, a phosphate buffer, a sulfonic buffer, raffinose, gluconate, histidine, and antioxidants [41]. Several experimental studies have shown that the Institute Georges Lopez-1 preservation solution (IGL-1) provides a good quality of preservation for kidney, intestine, pancreas and liver transplantation [42]. IGL-1 is an emerging extracellular-type electrolyte solution, low in viscosity, containing polyethylene glycol (PEG) 35 as a colloid. Finally, Pizarro et al. proposed the use of BG35 solution containing also PEG35 and gluconate. They showed that it did not affect ammonia metabolism of cold -preserved livers maintaining the physiological and biochemical hepatocyte functions [43].

Currently, UW is considered the "gold standard" for preservation of abdominal organs in the clinical field and is widely used. This PS effectively protects the kidneys, pancreas and liver but is less efficient in terms of integrity and function preservation in intestinal transplantation.

In conclusion, the search for the optimal preservation solution for organ transplantation requires further investigation in experimental models in order to meet the physiological demands of different grafts during preservation and to mitigate IRI.

2.3. Machine perfusion: The most common technique for organ preservation remains static cold storage. This technique exposes allografts to significant temperature fluctuations and is not entirely effective in attenuation of ischemic damage. This limitation motivated the development of new preservation techniques, including dynamics normothermic and hypothermic preservation performed by machine perfusion.

Normothermic machine perfusion (NMP) is based on emulating physiological organ blood perfusion. Normothermic recirculation attempts to reduce the severity of IRI by restoring

tissue perfusion and preventing anaerobic metabolism (44). Despite that machine perfusion is an alternative to static cold storage preservation the relationship between cost-effectiveness is still subject of discussion. The potential benefits of normothermic machine perfusion are numerous. The continued supply of oxygen and energy substrates allows an aerobic metabolism. Moreover, there is a continued washout of toxic metabolites. Experimental data suggested that NMP is an alternative and superior preservation method for solid organs such as kidney, liver, lung and heart [45]. On the other hand, hypothermic machine perfusion (HMP) has been shown to improve outcomes in organ transplantation such as kidney and liver In renal transplantation, HMP showed beneficial results in short and long-term graft function [46]. In a meta-analysis study O`Callaghan et al. showed that HMP reduces delayed graft function compared with static cold storage [47]. However, no difference was observed in primary non-function, acute rejection, long-term renal function or patient survival. At present there is some clinical evidence on the role of HMP in liver transplantation showing a reduction in early allograft dysfunction and shorter hospital stay. HMP also reduced molecular markers associated with IRI such as intracellular adhesion molecule 1 and TNF-a [48].

3-Actions performed on the transplant recipient:

Some interventions proved being productive when used during graft reperfusion in the recipient [49]. In 2003, Zhao et al. reported the effect of ischemic post-conditioning (IPOC) on IRI. IPOC consists in performing one or more short cycles of reperfusion/occlusion before allowing reintroduction of irrigation after prolonged ischemia phase. These authors showed that the IPOC was as effective as IPC to mitigate IR damage [50]. The mechanisms of action of IPOC have not been completely disclosed yet, however, as in IPC, it is believed that activation of adenosine receptors would be related to the protective effect. It has been shown that IPOC is able to reduce oxidative stress by improving the entry of O_2 to ischemic tissue [51]. In a recent clinical study, Kim et al. showed that IPOC promoted the recovery of graft function within the first 24 hr but did not affect the long-term graft function in kidney transplantation [52]. In the experimental field it has been reported that IPOC can reduce IRI of graft recovered from non-heart-beating donors and preserve function by reducing ROS and inhibiting apoptosis and inflammation in rat lung transplantation [53].

There is increasing evidence on the leading role of the complement system in IRI, therefore various therapies aiming to block different complement components are experimentally evaluated. The administration of antagonists of the C5a fraction as C5a antibodies were shown to be effective against IRI in experimental models. Though currently there is little information on the use of Eculizumab (an anti-C5 antibody) and Compstatin (a C3 inhibitor peptide) to mitigate IR damage, these anti-complement drugs appear also as promising therapies to decrease IRI [54, 55].

Concluding remarks

IRI constitutes a challenge for solid organ transplantation procedures. Minimizing IRI by a single or a combination of strategies described here may allow extending the ischemic time period in a TX procedure, which could provide a time frame for reaching better histocompatibility matching, and give the opportunity to organ procurement and allocation from distant geographic points. In the next few years we expect to witness the translation from many of the strategies discussed here to the clinical practice.

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Conflict of interest

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