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Review

Mechanisms of Cold Preservation and Reperfusion Injury for Solid Organ Transplantation: Implications for Partial Heart Transplantations

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Abstract: Cold preservation is a key component to organ procurement and transplantation. Cold preservation functions by slowing metabolic activity of procured organs and begins the period known as cold ischemic time (CIT). Reducing CIT and warm ischemic time (WIT) are paramount to minimizing donor organ damage from ischemia and the build-up of waste products and signals that drive reperfusion injury prior to transplantation into a matching recipient. Preventing damage from CIT and WIT and extending the amount of time that organs can tolerate has been a major goal of organ transplantation since donors and recipients are frequently not located within the same hospital, region, or state. Meanwhile, the amount of CIT that a transplant center is willing to accept differs based on the organ, the institution receiving the organ offer, and the doctor receiving the offer for that institution. With the introduction of a partial heart transplantation conducted last year at Duke University, it is important to discuss how much CIT transplant centers conducting a partial heart transplantation (pHT) are willing to accept. This article will review the physiology of WIT and CIT, associated organ damage, CIT variation among transplant centers and organ types, and provide a brief discussion of the future of pHT-accepted CIT and the need for research in this field.

Keywords: partial heart transplant; ischemia and reperfusion injury; heart valves; congenital heart disease; valve replacement; cold preservation

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1. Introduction

A primary concern for a solid organ transplantation is the cold preservation time or cold ischemic time (CIT) because donors and recipients are not typically co-located. There are 104,094 patients on the transplant waiting list in the U.S., but only 46,326 organs were transplanted in 2022 due to the lack of organ availability nationwide [1,2]. To close this massive gap, greater efforts must be made to increase organ availability, improve procurement and preservation protocols, and decrease the number of organs that are discarded.

Early in the field of transplants, hypothermic preservation was shown to significantly improve the amount of ischemic time that an organ could endure before transplantation [3]. The most common preservation technique used for transplanted solid organs in the United States is suspension of the procured organ in static or machine-perfused conditions, between 0–8 °C, with two major exceptions in the case of the normothermic perfusion machine's preservation of hearts and livers [4]. CIT is the amount of time that elapses after a cold preservation solution is introduced to the donor or donor organs following cessation of the donor's heart and anastomoses into the transplant recipient, as long as the temperature

of the organs is maintained between 0–8 °C. Alternatively, WIT is the amount of time that elapses above hypothermic temperatures (8 °C) and is more harmful than CIT in most conditions. WIT can accrue following extubation of a donation after a circulatory death donor, between preparing the organ on the back table to transplanting it into the recipient, during any point of the procedure where circulation or tissue oxygenation of the donor are not optimal, or any other conditions that leads to warmer-than-optimal ischemic conditions. Procurements frequently require multiple teams from different transplant centers being transported to a hospital that is different than the location of the transplant team's organ recipient. This can lead to an extended WIT and CIT of the procured organs, which can cause hypoxia-induced damage and oxidative reperfusion injury following transplantation and organ reperfusion [5]. Ischemia and reperfusion injury (IRI) have been major obstacles for transplantation and continue to cause graft dysfunction and failure [6]. Reducing CIT is a major goal for transplant centers and organ procurement organizations with the end goal of preserving organ viability. Organ viability can be defined as the functionality and restorative capabilities of an organ following procurement and transplantation with viability being influenced by CIT, WIT, donation type (circulatory, brain-dead donor, or live donor), presence of donor-specific recipient antibodies, sensitized-patients due to prior transplants and blood transfusions, and donor organ dysfunction acquired prior to procurement [7]. Organ injury from extended CIT is tissue-dependent with some organs, like kidneys, being highly tolerant to CIT, and cardiac muscle being very sensitive to CIT.

There is a wide variation across transplant centers on what donor organs they are willing to accept based on CIT, WIT, donor organ status, recipient status, and other transplant center specific criteria. Practice variation is partially due to accepted ischemic times being poorly understood, since generally it is accepted that the less WIT and CIT that an organ succumbs to, the lower incidence of tissue damage and graft rejection that may occur. Additionally, practice variation occurs due to variability among transplanting surgeons and the "aggressiveness" of a transplant center. In this ambiguous milieu, and with the recent introduction of a partial heart transplantation (pHT), it is of value to discuss the mechanism of CIT and WIT and how it may influence outcomes for a pHT. This paper aims to discuss the physiological basis of cellular damage due to CIT, CIT variation among different organs, and how CIT/WIT may play an important role in the value of a pHT and procurement of heart valves [8].

2. Physiology of Ischemia and Reperfusion Injury

2.1. Metabolic and Biochemical Basis of CIT and WIT

Ischemia is a state of insufficient blood flow to an area of tissue due to obstruction, disrupted gas exchange, or hemostasis [9]. Ischemia can lead to injury through a multitude of immunological, metabolic, and reactive oxygen species forming mechanisms (Figure 1) [10]. Ischemia injuries are a major challenge for many fields of medicine as they can lead to irreversible, life-threatening damage to affected organs. Nationally, the definitive beginning of ischemic time varies. The time of cross clamp is widely accepted as the beginning of the ischemic time, while other centers use a definitive oxygen saturation percentage of 80% on room air or a specific systolic blood pressure range as the beginning of the ischemic time. The ischemic phase ends at the time of organ reperfusion following transplantation with sufficiently oxygenated circulation [11]. Following the donor asystole in a procurement, a cold cardioplegia and aortic flush are administered throughout the donor's body and ice is placed directly on transplantable organs to begin cooling the body to slow cellular metabolism, since ischemia immediately results in a buildup of metabolic waste products, such as carbon dioxide, lactate, and hydrogen ions.

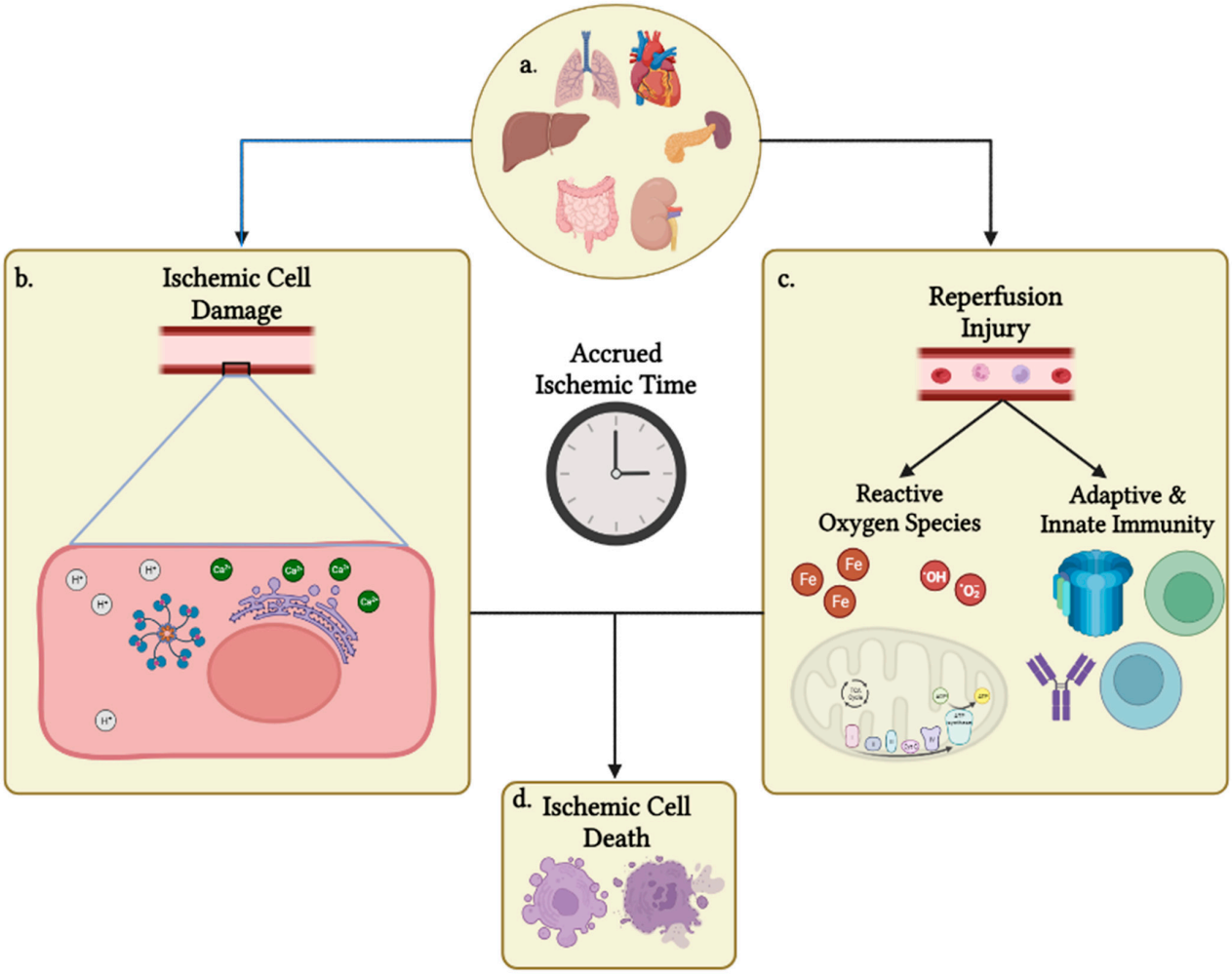

Figure 1. Overview of IRI affecting organs following procurement. (**a**) Solid organs that are currently able to be procured for transplantation. Each organ is unique in its susceptibility to cell ischemia based on a multitude of factors. (**b**) Organ ischemia begins a process of waste build up and dysregulation of cell homeostasis. Ultimately, the intracellular pH drops to deleterious levels, Ca++ dysregulation leads to calcium-induced apoptosis, and accumulation of cellular waste products and energy deficiency in the form of ATP depletion induces necrosis of cells. (**c**) Following transplantation, the donor organ is reperfused with oxygenated blood, which forms ROS and leads to the translocation of recipient-immune cells. Multiple mediators from both processes, such as T cells, B cells, complement proteins, iron ions, and the electron transport chain, further drive cellular and nuclear damage, as well as necrosis and apoptosis. (**d**) Processes listed in (**a**,**b**) both contribute to a reduction in donor organ viability via necrosis and apoptosis based on prior donor organ damage, the donation type conducted, CIT and WIT accrued, and recipient sensitization. Created with BioRender.com (accessed on 3 June 2023).

Initially following hypoxic exposure, ischemic cells begin to deplete intracellular ATP through normal biochemical activity, eventually halting the action of Na+/K+ ATPases, membrane Ca++ ATPases, and endoplasmic reticulum Ca++ ATPases. This results in increased levels of intracellular sodium and calcium, which causes cellular swelling and increased extracellular potassium. Increased hydrogen and sodium ions inside the cell lead to a decreased activity of Na+/H+ exchangers, trapping sodium and hydrogen inside of the cell. The increased level of H+ lowers the pH of the cell, potentially leading to impaired enzymatic activity, ribosomal dysfunction, and clumping of nuclear chromatin [12]. Additionally, the increased cytoplasmic sodium slows the activity of Na+/Ca++ exchangers, further exacerbating intracellular Ca++ levels [13,14]. High levels of intracellular Ca++

activate Ca++-dependent proteases, which damage intracellular structures, compromise membrane integrity, and increases the permeability of mitochondria, further impairing ATP production while releasing pro-apoptotic proteins [15]. Therefore, many cells are exposed to an acidic environment acutely, but prolonged ischemia accrued over the procurement process eventually leads to apoptosis, autophagy, or in severe damage, necrosis [16]. Apoptosis is a non-inflammatory, programmed-cellular death that is induced by a toxic accumulation of reactive oxygen species (ROS), cellular toxins, and metabolic waste products, as well as by dysregulated calcium within the cell and acute or chronic hypoxia. Necrosis is more common than apoptosis in IRI and is defined as cell death due to irreversible cell damage either by membrane disruption or from extreme organelle swelling, leading to decreased organ viability [17]. Necrosis is a major driving force of inflammation after reperfusion of the newly transplanted organ that stokes and further precipitates the immunological role of IRI [18].

2.2. Reactive Oxygen Species Generation and Damage

Hypoxia not only leads to organ damage through metabolic processes of ATP depletion, but states of hypoxia also lead to a buildup of intermediate products and a depletion of regulatory processes that can cause severe damage to the organ following reperfusion, known as reperfusion injury [19]. One major mechanism of damage in IRI is the production of ROS'. ROS' are oxygen-derived products with extra, unpaired valence electrons that are highly reactive with surrounding cellular components. ROS' are produced in stable, physiological processes for energy production, immune defense, and the degradation of cellular waste products like xanthine in the ischemic environment. ROS' are usually cleared by antioxidants available in the normal environment like NADPH, glutathione, tetrahydrobiopterin, and enzymes, glutathione peroxidase, superoxide dismutase and catalase [20]. ROS' cause damage in the body by the ROS' unpaired electrons reacting with surrounding amino acids, lipids, nucleic acids, and nitric oxide, or, importantly, by producing more ROS' in the presence of iron through the Haber–Weiss and Fenton reactions [21]. ROS stripping of electrons from surrounding structures disturb their physiological functions by disrupting membrane permeability, impeding enzymatic function, and by damaging DNA. There are a few major pathways which lead to the production of oxidative stress and damage, including the production of xanthine oxidase from AMP, the NADPH oxidase pathway, iron-dependent reactions, the electron transport chain (ETC), and the nitric oxide system all in the absence of antioxidant agents, such as reduced glutathione and tetrahydrobiopterin that are depleted prior to or following reperfusion [12,22].

2.2.1. Xanthine Oxidase Pathway in IRI

In the xanthine oxidase pathway, the ischemic environment causes energy depletion through the conversion of ATP to AMP. AMP is further converted to hypoxanthine by xanthine oxidase and produces superoxide as a byproduct. Xanthine oxidase can further break down hypoxanthine into uric acid as a waste product and an additional molecule of superoxide. The superoxide waste products made in the hypoxanthine metabolism can only be formed once the organ is reperfused with oxygenated blood and is not a direct consequence of WIT or CIT, but hypoxanthine does accumulate during these ischemic periods [23,24]. The superoxide formed from this process and other forms of ROS that form during ischemia and reperfusion that escape antioxidant fixation lead to a free radical attack, causing lipid peroxidation and nuclear damage due to reactive free radicals, further reducing organ viability post-transplant [23–25]. Hypoxanthine and xanthine oxidase's role in IRI have been targets of therapy for many years and led to the addition of allopurinol and glutathione to most preservation solutions used during the procurement process [26].

2.2.2. NADPH Oxidase Pathway in IRI

The NADPH oxidase pathway has increased activity and expression in the environment of IRI and is a major producer of ROS. NADPH oxidase is composed of seven isoforms

that differ in tissue localization, which consist of five NOX enzymes and two dual oxidases. Each NADPH oxidase uses oxygen as an electron acceptor and ultimately forms two molecules of superoxide per reaction [25]. The most well-described isoform of NADPH oxidase is found primarily in phagocytic cells and produces ROS to aid in the host's defenses against bacteria, but NADPH oxidase enzymes also participate in differentiation, endocrine signaling, cell growth, proliferation, apoptosis, cytoskeleton regulation, and migration of tissues [27,28]. Since NADPH oxidase enzymes are integral to cell function and regulation and two molecules of superoxide are made per oxidase reaction, NADPH oxidase is an important driver of ROS production and IRI.

2.2.3. Iron-Dependent Mechanisms of ROS Production

It has become an increasingly important topic of interest to target iron-dependent mechanisms of ROS production. As mentioned earlier, free iron molecules are a large source of ROS via the Haber–Weiss and Fenton reactions, which are both reactions forming highly reactive hydroxyl radicals in the presence of iron and hydrogen peroxide [21]. While these reactions are important to free radical formation of superoxide and hydroxyl radicals, it is believed that iron-dependent IRI is due to intracellular redox-reactive mechanisms and is not due to increased concentrations of circulating ROS [29]. The mechanism driving this damage appears to be, in part, due to hypoxia-inducible factor proteins (HIF). HIFs are transcription factors that increase cell survivability or lead to programmed cell death in a state of hypoxia by regulating anaerobic metabolism, angiogenesis, and apoptosis. HIFs in the setting of IRI, however, appear to also overexpress transferrin receptor 1 (TfR1), causing a cellular iron overload and oxidative injury. Iron overload and intracellular damage in the setting of IRI is also mediated by aberrant function of intracellular iron-binding proteins, membrane transporters, and proteins involved in iron processing and metabolism [21].

The hypothesis of intracellular iron being the driver of IRI is currently supported by research into the addition of iron chelators in cold preservation storage solutions, such as membrane-impermeable deferoxamine and membrane-permeable LK-614, with membrane-permeable iron chelators alone showing a significant reduction in IRI while membrane-impermeable iron chelators alone do not show a significant reduction in IRI. There are controversial results on the benefits of using both membrane-permeable and membrane-impermeable iron-chelators, with Radovitis et al. showing that the combination of membrane-permeable and membrane-impermeable iron chelators had similar results to groups treated with the membrane-permeable iron chelator alone [29,30]. A phase III clinical trial from 2020 using Custodiol-N, a cold preservation solution variation of Custodiol that has alterations to the recipe to decrease IRI including iron chelators deferoxamine and LK-614, showed a significant improvement in hypoxic injury, reduction of cold-induced IRI, and alleviation of adverse events following a warm solution exposure when used for kidney, liver, and kidney–pancreas procurement [31]. Additionally, a more recent paper from 2022 showed an improved preservation efficacy when Custodiol-N was used versus the original Custodiol recipe in a canine model of an orthotopic heart transplant [32]. A substantial body of data has shown that regulating iron levels in the setting of preservation improves outcomes in multiple organ models by maintaining and ameliorating ROS damage, but few centers have adopted this novel method of preservation.

2.2.4. Reactive Nitrogen Species and the Electron Transport Chain in IRI

The presence and production of nitric oxide (NO) is protective against ischemic damage under normal circumstances. However, due to the accumulation of ROS from other metabolic processes during the preservation process, ROS' have a greater opportunity to react with circulating NO, forming reactive nitrogen species, like peroxynitrite. Ultimately, these reactive nitrogen species can damage cellular components very similarly to the pathways used by ROS [14,33].

A final important producer of ROS following reperfusion is through the ETC. In the presence of oxygen, most cells produce most of their ATP to drive enzymatic and

biochemical reactions through the ETC pathway. The ETC is a series of mitochondrial enzymes that are initiated by the acceptance of electrons from NADH and FADH2, which were formed from other catabolic processes. Those donated electrons are transferred across a series of proteins with an increasing reduction potential, driving hydrogen ions into the intermembrane space of the mitochondria and producing a proton motive force that drives ATP synthesis. The transfer of these electrons can only occur in the presence of oxygen, which understandably increases the likelihood of producing ROS. The ROS formed in this pathway shortly after exposure to oxygen primarily comes from premature leakage of electrons from Complexes I, II, and III, and Coenzyme Q [34]. Understandably, reperfusion of oxygenated blood after a sustained period of ischemia can lead to a substantial increase in energy production through the ETC and resulting production of ROS.

2.3. Innate Immunity and Complement in IRI

In addition to metabolic imbalances, the immune system is a large function of ischemic injury and post-transplant rejection. These immune-mediated responses are the basis for immunosuppression in patients post solid organ transplant, as early inflammatory signals drive important downstream processes such as the recognition of foreign-donor antigen. Local cell death following procurement in the warm and cold ischemic phase leads to an immunogenic, yet sterile environment. Damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) are highly expressed in the transplanted organ and recipient following the transplant procedure. As ischemic time continues, DAMPs and PAMPs continue to accumulate. Immediately following reperfusion, toll-like receptors (TLR) and other damage or pathogen recognizing receptors interact with present DAMPs and PAMPs to create an inflammatory environment through the nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways [9,20,35]. Since MAPK and NF-κB are major drivers of immunological processes in transplant and many other fields, they are common targets for pharmacological and biochemical research. Animal models using TLR-4 knockout mice and siRNA NF-kB inhibitors have been shown to have a protective role and can prolong kidney survival, respectively, but this result has not been tested in other solid organs. NF-κB and MAPK can signal for significant inflammation in the transplant recipient post-reperfusion and can lead to a highly immunogenic environment that promotes the migration of antigen-presenting cells to activate lymphocytes, followed by the translocation of macrophages, lymphocytes, and neutrophils to the donor organ. ICAM-1 and VCAM are upregulated on the apical side of endothelial cells by inflammatory signals, such as IL-1 and TNF-a, to promote the adhesion and release of TNF-a, IL-1, and IL-8 for proliferation of immune mediators and for translocation and chemotaxis to the inflamed tissue [36]. These events precipitate cell death, activate complement, drive thrombosis, and increase vascular permeability, ultimately further driving rejection.

The Complement System's Role in IRI

In the presence of the inflammation stoked in the early stages of innate immune activation, circulating complement proteins are activated by cleavage to opsonize foreign antigens, bind cells tagged by alloantibodies, release more inflammatory signals, and activate the complement's membrane attack complex (MAC) to damage more cells through the classic, alternative, and mannose-binding lectin pathways [37,38]. The three complement pathways are initiated by different signals, but ultimately result in a universal pathway of chemokine signaling and MAC activation.

The C1 complement protein is required to initiate the classical complement pathway by binding to two adjacent antibodies bound to the target antigen [39,40]. Once bound, C1 is activated and assembles the C1 complex, which functions by cleaving and activating C2 and C4 to C4b2a, also known as the C3 convertase. The C3 convertase is ubiquitous in all three complement pathways and converts C3 to C3a and C3b. C3a serves as a chemokine, stimulating inflammation, while C3b binds either Factor B, the initiating protein in the

alternative pathway, or C4b2a, made in both the classical and lectin pathway to make C5 convertase. C5 convertase then converts the C5 protein to C5a, another chemokine, and C5b, which initiates the MAC assembly through the C6–C9 recruitment [40]. This entire process leads to further inflammation following reperfusion due to the complement chemokines C3a and C5a, in addition to cell lysis mediated by MAC. Since complement pathways are upregulated in donor organs following transplantation, there has been significant work exploring the benefits of blocking these pathways in animal models and clinical trials [37].

C1 inhibitors were considered promising targets, theoretically blocking classical pathway activation and damage in the presence of alloantibodies while preserving the alternative and lectin-binding pathways against infection. However, in a randomized controlled trial, the C1 esterase inhibitors given to patients at risk of IRI showed minimal-to-mild efficacy in reducing graft failure. Additionally, C1 inhibitors and anti-C1 antibodies were not significantly effective in treating antibody-mediated organ rejection, indicating that the classical pathway cannot be inhibited in isolation for effective prevention of graft rejection [41–43]. As C3 and C5 proteins are universal in the activation of all three complement pathways, they are currently the most promising targets of complements to reduce rejection following reperfusion. Blocking the complement at the C3 level has been hypothesized to have greater advantages over a C5 blockade due to an earlier termination of intermediate product formation in all three pathways [44–46]. Indeed, a recent primate-model using the C3 inhibitor, Cp40, inhibited antibody-mediated rejection via donor antigens [46]. This is a promising addition to the complement's role in reperfusion injury and rejection, and more research must be done to elicit the C3 inhibitor's efficacy in preventing associated damage in human subjects. While a significant amount of research has gone into understanding the innate immune system's role in the progression of ischemia and reperfusion injury, it is imperative that more information is elicited to apply these basic science principles to improving transplant patient outcomes.

2.4. Adaptive Immunity in the Physiology of IRI

The adaptive immune system plays a key role in organ rejection, and the creation of immunosuppressants that mitigate the adaptive immune system, such as cyclosporine, tacrolimus, prednisone, and other immunomodulators, were imperative to the success of organ transplantation. The adaptive immune system has some overlap with the innate immune system, but it is generally considered to be any immunological process or response that is modulated and improved with repeated exposure to foreign antigens. The main cell types of the adaptive Immune system are the lymphocytes, T cells, and B cells. T cells can be further described as cytotoxic T cells or helper T cells. Cytotoxic T cells, or CD8+ T cells, recognize host cells that have been invaded by foreign antigens—or, in the case of transplantation, recipient T cells recognize donor antigens on the transplanted organ's cell surface. Helper T cells, or CD4+ T cells, are integral to organ rejection and primarily modulate the immune response by releasing cytokines and activating other immune mediators. CD4+ T cells have been shown to appear in the donor organ, respond to, activate, and produce IFN-γ within 12 h of transplantation in mice models of liver, kidney, lung, and intestine transplantation [47].

2.4.1. T Cells in Reperfusion Injury following CIT

T cells play a major role in rejection following donor organ transplantation through antigen-dependent and antigen-independent activation [48]. CD4+ T cells have repeatedly been shown in animal studies to play an integral role in facilitating donor organ inflammation and damage following transplantation through cytokine secretion and a CD154 and CD40-mediated process [49,50]. CD4+ T cell activation leads to differentiation into Th1, Th2, Th17, and Treg cell lineages, which each play unique roles in the immune response [51,52]. Th1 and Th17 have been shown to cause a pro-inflammatory environment in the post-transplant milieu promoting tissue damage and rejection. On the opposite end of the spectrum, Th2 cells have been associated with inflammation inhibition, at least in

part by blocking the action and differentiation of Th1 cells [53]. Increased differentiation into the Treg cell (Foxp3 expressing T cells) lineage has shown evidence of improved graft survival rates. Additionally, when Tregs were recovered from normal syngeneic mice and implanted into T cell-deficient mice following an organ allograft transplantation, animal survival was significantly improved [54–56].

In addition to the CD4+ T cell lineages, CD8+ T cells contribute to IRI by the recognition of alloantigens on MHC class I of donor cells and initiates subsequent cytotoxic killing. While CD8+ T cells have been shown to be important to early donor organ damage and rejection, cell-mediated donor damage is limited by CD4+ T cell activation, and CD8+ T cell tolerance appears to form over time. While CD8+ T cells play a role in IRI, evidence of CD8+ T cell cytotoxicity in late-stage damage of donor allografts is still lacking evidence. In addition, cytotoxic T cell depletion by anti-CD8 antibodies in multiple animal models have not been protective against acute rejection [57,58]. While the information surrounding T cells and their effects in organ preservation and transplantation is promising, as well as the foundation of immunosuppression therapy in transplant, it comes at the cost of universal suppression of the patient's immune system, making them susceptible to severe infection and sepsis. To improve immunotherapy in transplanted patients, a more thorough understanding of pertinent cytokines' effects, crosstalk between cells, and the role of co-stimulation between immune mediators will need to be provoked before there will be a meaningful impact on recipient outcomes and livelihood.

2.4.2. B Cells in Reperfusion Injury following CIT

B cells are heavily involved in the immunological response following ischemia and reperfusion by producing donor-specific antibodies or alloantibodies following organ reperfusion. In the context of transplant, if T cells are absent or if the T cell costimulation of B cells is inhibited, donor-specific antibodies are not able to be formed [59–61]. The humoral mechanisms proven to drive acute organ rejection are anti-donor immunoglobulin-binding with subsequent complement-mediated cell lysis, antigen-dependent cell-mediated toxicity by natural killer cells, leukocyte recruitment, and vascular thrombosis due to the inflammatory activation of coagulation intermediates and immune-complex deposition [59]. While acute damage is a contributing factor to total organ rejection, the chronic effects of B cell activation are proposed to be the main drivers of late-stage allograft rejection as T cell mediated rejection and damage starts to dwindle within a year of transplant, while anti-donor antibodies continue to be formed by plasma cells following T cell tolerance [62]. CD4+ T cells, CD8+ T cells, Treg cells, and B cells have all been shown to play key roles in the progression of donor organ damage following transplantation, leading to the universal use of immunosuppressants, such as tacrolimus and cyclosporine. While the use of these drugs has made allograft transplantation possible, our understanding of the adaptive immune system's contribution to transplantation are still poorly understood. Eliciting these pathways and curating more targeted therapies is paramount to reduce off-target effects and to salvage the patients' overall immunity.

The hypoxic organ environment created during procurement, metabolism of donor tissues, production of ROS, and the immune reaction of the two environments brought together, work in tandem to cause IRI. It is a primary interest to modulate these circumstances to expand viable organ ischemic times and to produce an environment that reduces CIT/WIT damage and the subsequent effects of reperfusion injury. Advances to minimize the effects of CIT/WIT and reperfusion injury are essential for reducing recipient complications and ensuring that the sparse availability of transplantable organs are not squandered.

3. Variation in CIT/WIT among Solid Organs

Kidneys are the most transplanted organ in the United States, with 19,189 kidneys transplanted in 2021 [2]. One benefit leading to the large number of kidney transplants is the amount of CIT that kidneys can endure before they are non-viable. The CIT that is quoted

for most centers is less than 24 h, however with machine perfusion, some centers are willing to accept deceased donor kidneys with up to 40 h of CIT [7,63]. Since kidneys are relatively resistant to CIT/WIT, this allows for there to be a greater distance between a procurement center and potential transplant centers, improving the quantity of potentially interested centers and offering a greater opportunity for transplant centers to accept an aggressive offer. Additionally, kidney machine perfusion is a common practice used nationally with years of use and data to extend CIT and reduce delayed graft function. With this positive outcome, it is highly desirable to expand the amount of CIT that other non-kidney solid transplantable organs can endure. The current recommendations of CIT that organs can endure before transplantation are regularly discussed in the field. Variation may occur from center to center, but most transplant centers will accept CITs of four-to-six hours for orthotopic heart transplants, four-to-six hours for lungs, less than twelve hours for liver and pancreas, and six-to-eight hours for small intestine transplantation [63–68]. These accepted CITs are generally upper limits, and for the best patient outcomes, organs should be transplanted as soon as possible following procurement. Many of these non-kidney CIT tolerance times are with the use of hypothermic static preservation, but in recent years there has been a surge in availability of perfusion pumps for donor livers, hearts, and lungs.

As discussed earlier, there are many biological factors restraining the CIT and WIT that transplant centers are willing to accept. However, there are additional subjective and situational factors that must also be considered. First, CIT tolerance and transplant center acceptance can be heavily influenced by specific transplant center or transplant surgeon variation, which is a major source of ambiguity. Additionally, since CIT/WIT can cause cumulative damage to the donor organ, transplant centers with a very ill recipient or that are offered organs from a donor with marginal organ function are less likely to accept or transplant an organ that will succumb to more CIT/WIT due to the increased risks of complications. A final important point influencing CIT acceptance is a lack of sufficient data on CIT/WIT organ tolerance. For instance, the amount of CIT that lungs can endure has been a point of controversy within the field as some studies report an increased post-graft dysfunction and first-year mortality with CIT extending past 6 h, while studies like Ahmed et al. reported no significant changes in short- or medium-term outcomes of lung transplant recipients following transplantation of a lung that had succumbed to 8–11 h of CIT versus lungs transplanted with less than 8 h of CIT [65,69]. A recent surge in technological advances, as well as research in the field of transplant, while great, will continue to drive the variation of CIT/WIT that transplant centers are willing to accept as differing methods and techniques such as normothermic liver perfusion machines and ex vivo lung perfusion machines begin to become more regularly used within the field.

In summary, organ CIT variation is due, in part, to personal variation among transplant centers and specialists, the complex physiologic milieu of organ preservation and transplantation, health status of the donor and recipient, efforts to keep CIT as low as possible, empiric evidence, technological advances, and due to some organs having controversial evidence for the amount of CIT that they can endure [65]. Due to controversial evidence for organs in the field, along with the recent interest in partial heart transplantation, it is important that more research is done to elicit the amount of CIT that poorly defined organs can endure, as this research would provide guidance on pHTs, but also provide a baseline for which to gauge the efficacy of improving tolerance to CIT.

4. Discussion

A pHT is a potential procedure that is of interest to treat congenital heart diseases involving the recovery and transplantation of a donor heart valve [8]. Typically, congenital heart diseases require either a full, deceased donor heart transplant, or a valve replacement with either a mechanical valve or a bioprosthetic cadaveric tissue valve homograft. A status 1A pediatric heart transplant recipient is on the waiting list for a median of 108 days due to very few donor hearts that are viable and compatible with the patient, and a 17–30% waitlist mortality is reported as a continuing problem in the field [70,71]. Alternatively, cadaveric

valve homografts are abundant enough and are the most optimal valvular replacement in children currently due to the low cellular viability and low immunogenicity. However, due to the tissue preservation process, homografts are unable to grow with the recipient and require multiple replacements, which is burdensome on the patient and increases the risk of complications. Mechanical valve replacements must also be replaced as the patient grows, and the smallest mechanical prostheses are still too large for many infants. Another issue with mechanical valves is that placing them in an infant requires lifelong administration of anticoagulation therapy, putting patients at a significantly increased risk of bleeding [72]. These obstacles to management of patients with congenital valvular defects make pHTs a promising procedure (Figure 2). A pHT starts with a standard organ procurement in a donation by brain death or potentially a donation by a circulatory death patient. A donor's heart that is compatible in size to the recipient would be removed following the cross-clamp or cessation of donor blood flow, and the aortic or pulmonic valve alone would be recovered for orthotopic transplantation, similarly to the bioprosthetic valve homograft implantation procedure. However, the preservation processing of the valve recovered during an organ procurement would be similar to that of orthotopic heart transplants, which would preserve cellular viability and allow the valve to grow with the patient once transplanted. Pediatric patients that receive a pHT would be managed on immunosuppression until they reach adulthood. Once they have reached adulthood, the patient will be given the option to discontinue immunosuppressants, making the transplant a homograft, or they can have their transplanted valve replaced for a mechanical prosthetic valve and begin anticoagulant therapy. At this time, a pHT has been focused on aortic and pulmonic valve replacement due to the technical difficulty of mitral and tricuspid procedures [72]. Further use of pHTs also serves a beneficial role to patients nationally by expanding the number of life-saving organs that can be recovered by using hearts that have marginal or non-viable heart muscle but healthy cardiac valves. While this is an exciting new opportunity in the field of transplantation, it is still unclear how much cold ischemic time will be tolerated by donor heart valves due to its novelty.

Heart valves are endocardial-lined, avascular structures primarily composed of connective tissue, abundant in collagen, elastin, and proteoglycans [73]. This makes heart valves a unique addition to the field of organ donation as their avascular composition should resist IRI and be more tolerant to CIT before becoming nonviable for transplantation. In addition, studies by Mitchell and colleagues have shown that aortic valves of orthotopic heart transplants are preserved without immunological damage even in the setting of full myocardial graft rejection [74]. Heart valves are considered immunologically privileged tissues, which should alleviate a lot of the damage associated with other organ transplants and IRI. While heart valves may have a benefit due to their immune privilege and avascular nature, heart valves are very susceptible to inflammation since they are unable to regenerate spontaneously [75]. This mixture of immunological privilege and avascular tissue makes a pHT a very safe and effective treatment that is resistant to IRI. As more research investigates the viability of pHTs, there will need to be continued research with the intent of establishing accepted cold ischemic times to reduce potentially irreversible complications. Due to valves having a close association with cardiovascular tissue and a general understanding that less CIT is better for organ viability, early use of this procedure will most likely see minimal CITs. Therefore, it is imperative to elicit the maximum CIT that maintains organ viability to expand the distance and availability for these life-saving organs to patients nationwide.

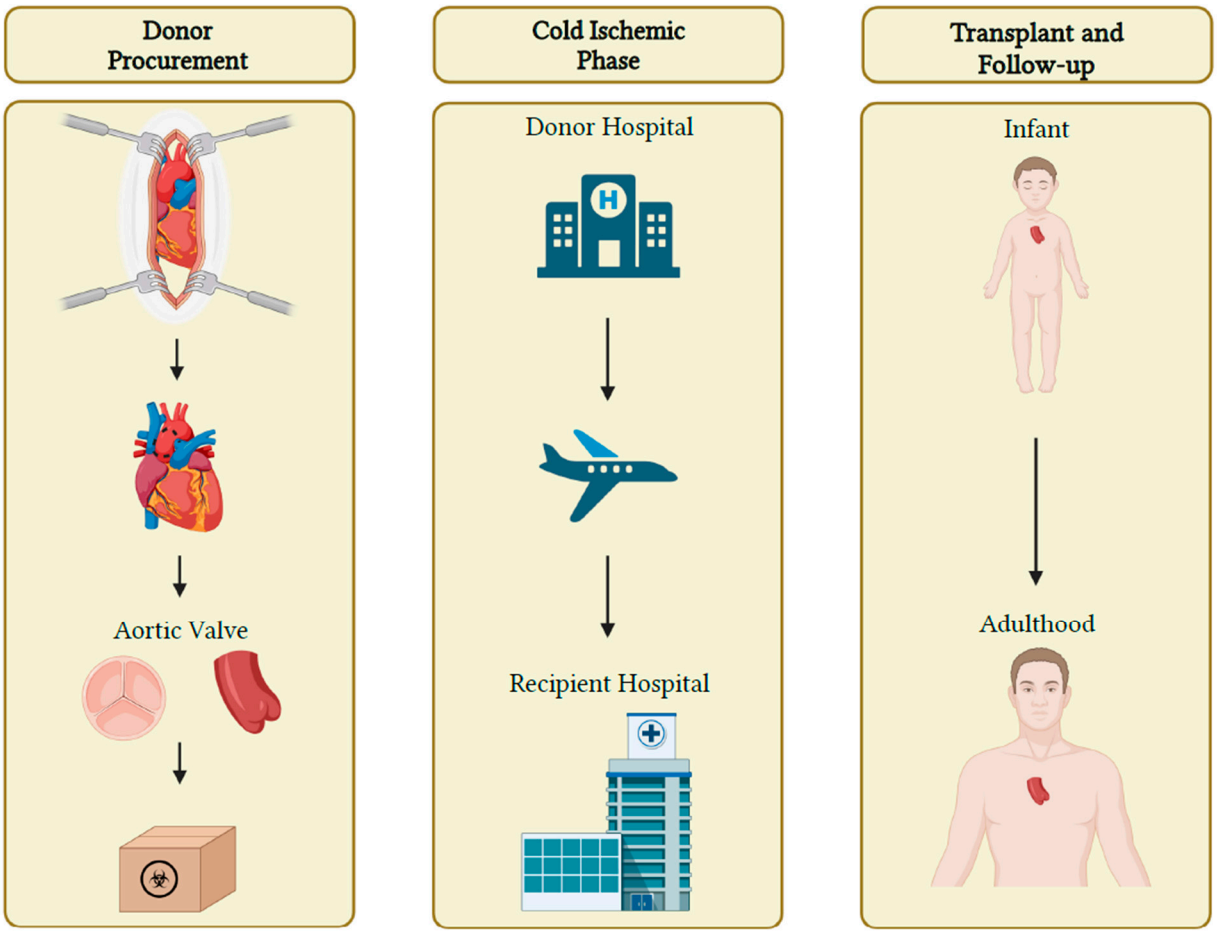

Figure 2. Overview of the partial heart procurement and transplantation process. During the donor procurement, the donor heart is removed and the aortic valve with a portion of the aorta is preserved and placed in a cooler full of ice for transportation to the recipient transplant center. The viable, donor aortic valve with a portion of the aorta is then transplanted into an infant to repair a congenital aortic valve malformation that grows with the recipient into adulthood. Created with BioRender.com (accessed on 3 June 2023).

5. Conclusions

Solid organ preservation is still a young and growing field that needs substantial advances to improve the number of available donor organs for recipients and to improve solid organ viability by mitigating cold and warm ischemic damage between donor procurement and recipient transplantation. Available research has shown that free radical damage, inflammation, immune mediators, ATP depletion, and calcium overload, coupled with a lack of available, viable donor organs, are major limitations for transplants in general, leading to complications and death for patients on the organ waitlist. Improving procurement practices and reducing donor organ damage are essential as the field moves forward. As a potential addition to the field of transplantation, a pHT is a promising procedure that expands the number of viable transplantable organs available and offers greater outcomes for many pediatric heart recipients who do not need a total donor heart, but a functional cardiac valve that grows with them into adulthood.

6. Future Directions

Expanding research on pHTs is essential to the future of this budding procedure. Testing IRI tolerance and establishing guidelines on accepted CIT based on other, current solid organ transplant guidelines are essential. Additionally, continuing research into the viability of valves following procurement and CIT is needed to introduce this remarkable

option for future patients. Due to the unique nature of heart valves being the only avascular transplantable organ so far, more research will need to be conducted to elicit the effects, if much at all, of CIT and IRI, what procurement practices to implement to ensure patient safety, and how patients will respond to this new procedure in these exciting times for transplants.

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Abbreviations

The following abbreviations are used in this manuscript:

CIT	Cold ischemic time
WIT	Warm ischemic time
pHT	Partial heart transplantation
IRI	Ischemia and reperfusion injury
ATP	Adenosine triphosphate
ROS	Reactive oxygen species
NADPH	Nicotinamide adenine dinucleotide phosphate
HIF	Hypoxia-inducible factor
ETC	Electron transport chain
NO	Nitric oxide
DAMPs	Damage-associated molecular patterns
PAMPs	Pathogen-associated molecular patterns
TLR	Toll-like receptor
MAPK	Mitogen-activated protein kinase
MAC	Membrane attack complex

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