Review

Mitochondria as Therapeutic Targets in Transplantation

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Advances in surgical procedures, technology, and immune suppression have transformed organ transplantation. However, the metabolic changes that occur during organ retrieval, storage, and implantation have been relatively neglected since the developments many decades ago of cold storage organ preservation solutions. In this review we discuss how the metabolic changes that occur within the organ during transplantation, particularly those associated with mitochondria, may contribute to the outcome. We show how a better understanding of these processes can lead to changes in surgical practice and the development of new drug classes to improve the function and longevity of transplanted grafts, while increasing the pool of organs available for transplantation.

Organ Preservation during Transplantation
Development of organ transplantation has transformed lives [1–10]. Initially using organs from living donors, its success led to a demand for organs from deceased donors, and thus a need for prolonged periods of extracorporeal storage to allow transport to the recipient center [1,2]. In situ flushing of donor organs with cold University of Wisconsin solution has become the most widely used procedure for organ preservation [1,2]. This approach is thought to protect the organ by slowing metabolism through cooling, providing osmotic support that stops cell rupture following disruption of ionic gradients as the ATP:ADP ratio falls, and reducing the ischemia–reperfusion (IR) injury that occurs upon transplantation of the graft (see Glossary) into the recipient [11,12]. Although emerging technologies allow preservation of some organs under normothermic and/or normoxemic conditions [13,14], rapid cooling at the onset of ischemia remains the mainstay of organ preservation, enabling the heart, kidney, and liver to be stored for up to ~4 h, >24 h, and >12 h, respectively (Box 1). The precise duration depends on the quality of the organ, with organs from young, healthy donors being better able to tolerate longer periods of cold ischemia. Despite its widespread use and importance for transplantation, our understanding of why cold storage is protective is incomplete, and our understanding of the critical metabolic changes that occur during organ retrieval, storage, and implantation in the recipient has lagged behind technical and immunological advances.

Recently there has been greater understanding of how mitochondrial metabolism contributes to cell damage in general [15–19], and to poor outcome in transplantation in particular [20–24]. We discuss the mitochondrial and metabolic changes that contribute to tissue damage during transplantation and consider how to target these pathways therapeutically.

How Mitochondria Contribute to Graft Dysfunction
The metabolic factors that impact on transplantation arise mainly because the organs to be transplanted are ischemic, often for long periods of time. In this review we focus on the general properties of solid organs; however, it is important to note that there are significant differences in ischemic metabolism between different organs. A clear exception is the lung, which is inflated

Highlights
The mitochondrial and metabolic changes that occur during transplantation have been neglected, compared with technical and immunological aspects. Knowledge of how these factors contribute to graft function may improve outcomes for patients.

Of particular interest is how ischemic metabolism depletes adenine nucleotides and leads to an accumulation of NADH and succinate. These processes are greatly enhanced by temperature, emphasizing the importance of rapid cooling of organs during ischemia.

The metabolic changes during ischemia prime the organ for damaging ischemia–reperfusion injury upon reperfusion. This leads to the release of damage-associated molecular patterns (DAMPs), such as mitochondrial DNA, that initiate an innate immune response, which can contribute to organ rejection.

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with oxygen before storage, enabling aerobic metabolism in the alveolar epithelium to continue during storage [25]. Unlike other organs, circulating blood removes oxygen from the lungs as opposed to supplying it to other organs, and the absence of circulation is not deleterious to an oxygenated lung. The heart is also a special case, where storage is preceded by the administration of cold cardioplegia, the hyperkalaemic composition of which ensures rapid cessation of contractile activity and thus energy consumption in addition to the metabolic effects of cooling [26,27]. Much of our knowledge of ischemic metabolism in the heart comes from studies on hearts held temporarily ischemic by cardioplegia during cardiac surgery.

During ischemia the lack of ATP production by mitochondrial oxidative phosphorylation leads to a gradual decrease in the ATP:ADP ratio which eventually causes cell death [22,28]. Then, upon implantation and reperfusion of the organ in the recipient, mitochondria contribute to IR injury to the organ similar to that which occurs during myocardial infarction and stroke [22]. Mitochondria are central to IR injury, generating damaging reactive oxygen species (ROS) upon reperfusion, which cause tissue damage [29,30]. Furthermore, the damage to mitochondria upon reperfusion releases damage-associated molecular patterns (DAMPs), which activate the innate immune response and enhance inflammatory damage to the tissue, and contribute to organ dysfunction and rejection [21,31,32]. Here, we consider the mitochondrial and metabolic changes during transplantation that contribute to graft damage, with a focus on the role of IR injury upon implantation.

Mitochondrial Metabolic Changes during Organ Retrieval and Storage
Ischemic Metabolism
During conventional organ transplantation, from living donors or from donation after brainstem death (DBD) or donation after circulatory death (DCD) donors, periods of ischemia are inevitable (Box 1). The lack of oxygen during ischemia means there is no ATP production by oxidative phosphorylation, thus the organs cannot rely on the metabolism of fat, ketone bodies, or amino acids to maintain the supply of ATP. Instead, during storage anaerobic glycolysis is essential to maintain a sufficient ATP:ADP ratio to keep cells alive (Figure 1, bottom left, Key Figure). During ischemia there is still continual consumption of ATP for essential basic cell functions, such as maintaining ion gradients. In addition, in the absence of oxygen the mitochondrial proton motive force will no longer be sustained through proton pumping by the respiratory chain; instead the F$_\text{F}_1$-ATP synthase will act in reverse, hydrolyzing ATP generated by glycolysis to pump protons across the mitochondrial inner membrane [28,33,34]. Although there is a protein, inhibitory factor-1, that can block this ATP hydrolysis in vitro and is activated at the decreased pH present during ischemia [35], its role in vivo is not well understood and mitochondrial ATP hydrolysis is still a significant drain on ATP during ischemia [28,33,34]. These processes together contribute to the gradual decrease in the ATP:ADP ratio during ischemia, causing a disruption in cell function and eventually cell death [28,36].

Glycogenolysis and Glycolysis
To maintain glycolytic ATP generation, the NADH produced by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) must be recycled to NAD$^+$ (Figure 1, bottom left). This is done by lactate dehydrogenase (LDH), which oxidizes NADH to NAD$^+$, reducing pyruvate to lactate. The lack of blood flow to the organ during ischemia means that the glucose required to drive glycolysis cannot be provided from the circulation and so instead comes from breakdown of its glycogen stores. If the organs cannot continue to utilize glycogen, they can no longer sustain their ATP:ADP ratio. This could in principle occur because of the consumption of all the glycogen stores. However, it often seems to be due to the inability to utilize these stores because of the inhibition of glycolysis at GAPDH caused by the high NADH/NAD$^+$ ratio and the low pH that arises during ischemia [22,28].

Glossary
Complex I: this mitochondrial respiratory complex normally oxidizes the NADH generated in the mitochondrial matrix with the associated reduction of ubiquinone to ubiquinol and pumping of protons across the mitochondrial inner membrane. Under some conditions electrons can move backwards through this complex, and this RET leads to ROS production.
Graft: a tissue such as an organ moved from one part of the body to another, or to another person.
Implantation: the process of connecting the replacement organ to the blood vessels of the recipient.
Ischemia: interruption of blood supply that prevents the delivery of the oxygen and substrates needed to maintain organ viability. In cold ischemia the organ has been cooled down to just above freezing while in warm ischemia the organ has not been cooled.
Mitochondrial permeability transition pore (MPTP): a large permeability pore that opens in the mitochondrial inner membrane during IR injury due to elevated ROS and calcium. This leads to cell death and also to the release of DAMPs such as mtDNA.
Perfusion: the process of passing a fluid through an organ. ‘Machine perfusion’ is where a machine continuously recirculates the fluid – preservation solution or blood – through the organ. ‘Perfusion in situ’ is where an organ is flushed with preservation solution while still within the body before removal.
ROS: reactive oxygen species. These damaging molecules are produced by mitochondria during IR injury that cause oxidative damage due to their high reactivity with other biomolecules.
Static cold storage: where organs are flushed with preservation fluid and placed in a bag of the same solution which is then stored on ice.
Succinate dehydrogenase (SDH): or complex II, is a member of both the respiratory chain and Krebs cycle. It oxidizes succinate to fumarate and thereby reduces ubiquinone to ubiquinol.
The generation of lactate from pyruvate thus acts as a safety valve to prevent a build-up of NADH to maintain glycolytic ATP production. Under anaerobic glycolysis, such as in fast twitch muscle or red blood cells, lactate is exported from the cell by monocarboxylate transporters (MCTs) to the circulation which at the same time pump protons out of the cell to counteract cell acidification [37]. The situation during ischemia is quite different, because in the absence of blood flow the concentration of lactate in the extracellular fluids rise and the pH drops, blocking efflux of lactate from the cell. Preservation solutions used for organ transplantation can counter the pH fall with phosphate or citrate buffers. However, the importance of lactate efflux from the cell is underappreciated. If lactate builds up in the cell then LDH will no longer be able to oxidize NADH to NAD⁺ and glycolysis will be blocked at GAPDH (Figure 1, bottom left).

Adenine Nucleotide Breakdown

Another important metabolic change that occurs during ischemia is the degradation of purine nucleotides (Figure 1, bottom right). The lack of oxidative phosphorylation leads to a persistent lowering of the ATP:ADP ratio and a consequent accumulation of ADP [36]. The action of adenylate kinase converts two ADP molecules to one ATP and an AMP, leading to the accumulation of AMP [28,36]. The accumulated AMP is then deaminated by AMP deaminase to inosine monophosphate (IMP) which is in turn dephosphorylated to inosine by a 5′-nucleotidase [36,38]. The inosine is then degraded to hypoxanthine by purine nucleoside phosphorylase. The hypoxanthine can then be further metabolized to xanthine and uric acid by xanthine oxidoreductase, although its variable tissue and species distribution and dependence on oxygen affects its contribution [36]. AMP can also be dephosphorylated by a 5′-nucleotidase to adenosine, which can be further deaminated by adenosine deaminase to inosine. Thus, during ischemia the accumulation of AMP leads to its degradation and because of its dynamic equilibrium with ATP and ADP all adenine nucleotides are severely depleted by ischemia. This lack of ADP diminishes the ability of substrate level phosphorylation to generate ATP.

Box 1. Current Procedures in Deceased Donor Transplantation (See Figure I)

Retrieval

Donation after brainstem death (DBD): following the declaration of death by brainstem criteria, the donor continues to receive cardiorespiratory support until the time of donation when in situ cold perfusion and topical cooling is used to rapidly cool organs.

Donation after circulatory death (DCD): following the withdrawal of life support death is declared by cardiocirculatory criteria. After a stand-off period, the donor is rapidly cannulated and the organs perfused with cold preservation solution before retrieval. More recently, normothermic regional perfusion (NRP, see later) has been used to minimize the effects of warm ischaemia during DCD.

Storage

Following retrieval, organs are stored for transport to the recipient. A range of techniques have been employed to minimize ischemia and ischemic injury during this period.

Hypothermic static preservation: organs are stored in ice-cold preservation solutions that have been designed to provide an extracellular oncotic force to minimize cellular swelling, a buffer to counter intracellular acidosis and electrolytes to maintain ion gradients.

Hypothermic machine perfusion: there has been renewed interest in the use of hypothermic machine perfusion, primarily in liver and kidney transplantation. It has been proposed that this has a beneficial effect by removing metabolites from the organs during storage and flushing capillaries. Some investigators have also oxygenated the preservation solution hypothermic oxygenated perfusion (HOPE). This has been shown to have promising outcomes in liver and kidney transplantation.

NRP: following cardiocirculatory death, the donor is rapidly cannulated and the organs perfused in situ with oxygenated blood. During this period organs can be assessed, and therapeutic drugs or measures to improve organ function can be administered. The organs are then retrieved using a similar technique to DBD retrieval. Cardiothoracic and abdominal organs (with the exception of intestinal transplants) have been transplanted successfully using this technique.

Ex situ normothermic oxygenated machine perfusion (NMP): this technique has also received widespread interest more recently with considerable investment into the development of technology for a range of organs to enable ex situ perfusion of the heart, lungs, liver, and kidneys. There is interest in the use of these technologies for a range of clinical situations including prolonging the period of time organs can be maintained ex situ to facilitate the transplant operation, assessment of organs prior to transplantation, reconditioning of organs, and as a potential platform for the delivery of agents. NMP can be used immediately following retrieval or following a period of hypothermic static preservation.
Succinate Accumulation

An emerging metabolic marker of ischemia is the accumulation of succinate (Figure 1, top) [29,30]. During aerobic metabolism electrons from many sources are passed on to the co-enzyme Q (CoQ) pool within the mitochondrial inner membrane. In the absence of oxygen, the highly reduced CoQ pool can then pass electrons onto fumarate by reversal of succinate dehydrogenase (SDH), generating succinate [29,38]. In addition, glutamine and glutamate breakdown to 2-oxoglutarate and on to succinyl CoA may continue during ischemia leading to the build-up of succinate as the highly reduced CoQ pool will prevent its oxidation to fumarate [39]. The fumarate for succinate generation likely enters mitochondria from the cytosol as malate in exchange for succinate via the dicarboxylate carrier, and within the matrix malate is converted to fumarate by fumarate hydratase [29,38]. The malate in the cytosol likely arises as a consequence of the highly reduced NADH/NAD+ pool that reduces oxaloacetate to malate by malate dehydrogenase. In addition, the accumulation of AMP and IMP during ischemia may activate the purine nucleotide cycle which generates fumarate which will be converted to malate by cytosolic fumarate hydratase [29,38,40,41]. Succinate accumulation is now recognized as a universal marker of ischemia [22,30,39,42–44], with its export to the cytosol decreasing the activity of 2-oxoglutarate dependent dioxygenases, such as prolyl hydroxylase-1 which is involved in the hypoxia inducible factor-1α hypoxia sensing pathway [45–47].
Figure 1. Mitochondrial metabolism, top. During ischemia the lack of oxygen prevents oxidative phosphorylation, blocking oxidation of many substrates and the lack of glucose leads the cell to switch to glycolysis to provide ATP. The mitochondria switch to ATP hydrolysis and the ATP:ADP ratio falls. The lack of oxygen also leads to a highly reduced coenzyme Q pool which leads to the reduction of fumarate to succinate by succinate dehydrogenase, and also an increase in succinate from glutaminolysis. Ischemia will also lead to the concentration of malate in the cytosol that will increase and exchange for succinate, enabling succinate to get to succinate dehydrogenase (SDH).

Glycolysis at glyceraldehyde-3-phosphatedehydrogenase (GAPDH) reduces NAD to NADH which is recycled to NAD+ by lactate dehydrogenase to maintain glycolysis. In the absence of lactate efflux from the cell through the monocarboxylate transporters during ischemia the NADH accumulates and the pH drops, decreasing glycolysis by inhibiting GAPDH, even if there is glycogen left. Cell death, bottom center. This leads to both a decrease in the ATP:ADP ratio and in the decrease in the concentration of all adenine nucleotides, disrupting cell function and ultimately leading to death. Adenine nucleotide breakdown, bottom right. The increase in ADP leads to a dramatic increase in AMP due to the action of adenylate kinase and the AMP is then broken down to adenosine or inosine monophosphate (IMP) and then degraded further.
Hence a major benefit of the cooling of organs during transplantation is that it slows metabolism and allows the organ to sustain a sufficient ATP:ADP ratio to defer cell death. In addition, preservation solutions aim to counteract changes in ionic distribution and provide osmotic support, thus enabling the cells to better survive the decreased ATP:ADP ratio. This cold tolerance is finite, because even though metabolism is slowed, it does not stop at low temperature, so ATP-demanding processes continue, leading to a delay, but not an absence of damage.

**Mitochondrial Metabolic Changes during Organ Implantation and Reperfusion**

Mitochondrial metabolism is critical during organ implantation and reperfusion within the recipient. During reperfusion of the ischemic tissue extensive tissue damage occurs, known as IR injury, which causes the morbidity and mortality associated with heart attack and stroke [48–51]. Paradoxically, the restoration of oxygenated blood to the ischemic tissue itself initiates the IR injury [29,52–55]. The cause of IR injury is a burst of the ROS superoxide from the mitochondrial respiratory chain upon reperfusion that initiates a cascade of tissue damage [29,54]. This process had long been tacitly assumed to be a random consequence of the reperfusion of ischemic tissue, however, recent studies suggest that IR injury occurs as a result of specific processes and is not just a catastrophic breakdown of cell function [29,56]. During ischemia, the metabolite succinate builds up dramatically, then upon reperfusion the accumulated succinate is rapidly oxidized driving superoxide production at complex I by reverse electron transport (RET) (Figure 2) [29,30]. This can cause oxidative damage to mitochondria, induce the mitochondrial permeability transition pore (MPTP) [57–59] and lead to cell death (Figure 2). Supporting the relevance of this model of IR injury for transplantation, it was recently demonstrated that succinate accumulation and oxidation contributes to IR injury and graft dysfunction in a mouse model of heart transplantation [22].

Mitochondrial damage caused by IR injury during organ implantation can contribute to graft dysfunction and loss in a number of ways (Table 1). Cell death and organ dysfunction caused by induction of the MPTP will ultimately lead to tissue scarring and re-modeling [29,60]. In addition, in response to cell death DAMPs such as mitochondrial DNA (mtDNA) are released by damaged mitochondria into the cell, which activate the innate immune response locally [61–63]. mtDNA can be released into the circulation and activate immune responses [21,24], as can metabolites such as succinate, which can go on to activate the SUCNR1 receptor and potentially enhance immune responses [64–66]. These inflammatory effects can contribute to organ rejection and long-term graft failure. Thus, the mitochondrial metabolic processes that occur during organ retrieval, storage, and implantation are greatly exacerbated by periods of warm ischemia (WI) and have a significant impact on immediate graft function and also on the long-term outcome.

**Pharmacological Strategies Targeted to Mitochondria during Transplantation**

How mitochondrial metabolism contributes to graft damage during transplantation suggests a number of possible pharmacological interventions to improve outcomes (Figure 3). We discuss general therapeutic approaches, rather than systemically reviewing specific drugs and trials. An important aspect of pharmacological interventions in transplantation is that there are several points to deliver a drug to the donor, isolated graft, or recipient. A drug could be delivered to the organ in the donor before the onset of ischemia. Although this is possible in DBD transplantation (where the legal diagnosis of brainstem death is made before organ donation), the current legal frameworks in most countries prevent treatment of the donor in DCD transplantation (where the diagnosis of death is not made until after circulatory arrest). In addition, the organ can be treated with drugs ex vivo, by supplementing the organ preservation solution used to flush the organ prior to storage, or it can be administered to the organ just prior to implantation. Finally, drugs can be given to the recipient before, during, or after implantation of the organ.
Drugs could be targeted to prevent metabolic changes during ischemia, for example, by slowing the degradation of adenine nucleotides by inhibiting xanthine oxidoreductase with allopurinol, or by adding hypoxanthine to the graft [36]. In addition, the suppression of glycolysis by NADH accumulation during ischemia may be achieved by enhancing the NAD⁺ pool by NAD⁺ precursors such as nicotinamide ribosides [67,68], or by enhancing NADH consumption by preloading with pyruvate. Finally, malonate is a potent inhibitor of SDH and its cell permeable form, dimethylmalonate (DMM) can decrease succinate accumulation during ischemia and thereby lower mitochondrial ROS production upon reperfusion [22,30].

It is also possible to add compounds just prior to reperfusion in the recipient to decrease mitochondrial ROS production. This could be done by stopping oxidation of succinate with malonates [69,70], or by adding compounds that interact with the respiratory chain to decrease ROS production, such as S1QELs [71], or the mitochondria-targeted S-nitrosating agent MitoSNO [72–74]. Downstream of ROS production during IR injury, it may also be possible to protect mitochondria from oxidative damage with antioxidants [75], and mitochondria-targeted antioxidants have shown protection against IR injury in a mouse model of heart transplantation [20]. Induction of the MPTP is major point of damage downstream of the ROS production by mitochondria upon reperfusion. Blocking MPTP induction by targeting the mitochondrial cis-trans prolyl isomerase cyclophilin D (CyD) with cyclosporin A (CsA) protects...
mitochondria during IR injury [57–59,76]. For example, when CsA was administered at the same time as reopening the coronary artery in a Phase II trial of heart attack patients it showed promising results [77]. However, when extended to Phase III [78,79], it was unsuccessful. This was possibly owing to the difficulty of delivering the CsA rapidly enough to the heart during reperfusion, but this constraint should not affect the administration of CsA to organs for transplantation. Furthermore, CsA is already widely used in transplantation, although it is administered to the recipient after implantation, rather than infused into the graft prior to reperfusion. The final stage of pharmacological intervention is to deal with the proinflammatory effects of the release of DAMPs such as mtDNA by damaged mitochondria and a number of drugs are being developed targeted to downstream inflammatory pathways [80].
Finally, although not strictly pharmacology, the role of glycogen in providing glucose within the organ during storage suggests that altered nutrition of the donor prior to retrieval could glycogen-load the organs [81], although this would need to be done in parallel with ways of preventing the inhibition of GAPDH by reduction of the NADH/NAD⁺ pool, so that the glycogen stores could be utilized.

**Box 2. How Much Warm Ischemia Occurs during Organ Transplantation?**

A corollary of the benefit of cooling during organ preservation is that warm ischemia (WI) is particularly damaging, and this is shown by the very rapid irreversible damage that occurs to organs during WI [22,28]. Although it is sometimes overlooked, there are inevitable periods of WI during transplantation [22]. In DCD there will inevitably be WI as organ perfusion can be severely reduced before the formal diagnosis of circulatory arrest, and during the period of circulatory arrest. Prolonged time to flush organs in situ, remove them from the donor and place them on ice has been demonstrated to contribute to graft failure [100–102], and WI time has been shown to more harmful than CI time on a minute-for-minute basis [103]. For DCD donors the current critical practice of a ‘stand-off’ period (typically 5 min) after cessation of circulation before circulatory death can be confirmed and the organs are retrieved, also makes periods of WI hard to circumvent. Even for living donors, there will be periods of WI during retrieval when the organ’s blood supply is stopped within the donor. Figure I shows a picture of a pig heart, which is about the same size as a human heart (~300 g), being flushed and stored in cold flush under exactly the same conditions as used for human heart transplantation. (Reproduced in part from [22].) During cooling the temperature at the surface and core of the heart can be measured using thermocouples. The graphs show that the temperature of the heart core cools slowly, hence even during conventional cooling the tissue takes many minutes to cool down towards ice temperature, thereby experiencing WI (Figure I) [22]. Finally, during implantation within the recipient, the organ is also exposed to WI as it is gradually warming up while its blood vessels are being connected to those of the recipient, before it is perfused with blood [22], despite best attempts at keeping it cool. Therefore, during conventional transplantation the tissue will often experience two periods of WI separated by prolonged CI. During these periods there may be cell death and damage, resulting in poorer outcomes [104–106]. In addition, even time periods as short as a few minutes of WI can lead to major metabolic changes, equivalent to those that take hours of storage under CI [22,29]. Although these alterations may not kill the tissue in themselves, these short periods of WI can lead to metabolic changes that impact on the subsequent IR injury and related damage which occurs to the tissue upon reperfusion in the recipient (see Table 1 in the main text). Hence the need for drugs that target aspects of mitochondrial metabolism during WI, since some WI is inevitable in most forms of transplantation.

**Figure I. Warm Ischemia During Organ Donation and Implantation.** Despite optimal procedures, it typically takes >10 minutes to adequately cool human organs, as illustrated by the data from cooling of pig heart. Therefore, both DBD and DCD donation lead to exposure of organs to warm ischemia.
Nonpharmacological Changes to Clinical Practice

Our growing knowledge of the metabolic changes that occur during transplantation provides an incentive to improve clinical practice. A key aspect that has emerged from our focus on mitochondrial and metabolic changes during transplantation is the importance of avoiding even short periods of WI (Box 2). This suggests we should explore new methods to cool organs more rapidly. There are physical limits to how quickly an organ can be cooled down by conventional flushing with ice-cold storage solution, but it is possible to accelerate this by optimizing current perfusion practices. Ideally, the organ would be cooled before it was exposed to ischemia during the retrieval process, for example, by perfusing with cold, oxygenated blood or preservation solution in situ within the donor. This may be possible in a DBD donor, using an adapted cardiopulmonary bypass circuit, but it is difficult to avoid WI in the DCD setting [82–84]. Another approach may be by cooling the donor during organ retrieval [85]. A further strategy to manage the WI that characterizes the dying process in DCD donation is by restoring an immediate oxygenated circulation to the organs using the donor’s own blood and an extracorporeal oxygenator and pump, a procedure called normothermic regional perfusion [86,87]. This has the advantage of allowing the ischemic organs to recover before imposing an immediate additional period of cold ischemia (CI), and offers the opportunity of manipulating the organs in situ during this period of reperfusion (e.g., adding protective agents) while still being oxygenated in situ and then removed and cooled [86,87]. A related approach that can be used in parallel with minimizing WI is to decrease metabolic activity during CI still further by supercooling the organs and storing them at temperatures below 0°C. This has been done for human livers which, by utilizing an ex situ perfusion to load the organ with antifreeze compounds to avoid ice formation, enabled extended storage at −4°C [88].

Until recently, cold static storage was the main method used to store organs between retrieval and implantation. Now there are a number of ways of continuously perfusing organs during storage (Box 1). Hypothermic oxygenated perfusion (HOPE), initially used in the 1960s [5], has received renewed interest. Hypothermic perfusion has the benefit of continued perfusion of the organ, supplying substrates and removing waste products such as lactate. It has also been suggested that a brief period of HOPE before implantation, where oxygen is supplied at low concentrations in the cold, either at a fixed hypothermic temperature [89–91] or during controlled rewarming (COR) [92–95], may dissipate the succinate build-up with minimal ROS generation while allowing regeneration of ATP. This approach has been shown in animal models to be associated with better outcomes for preserved livers and kidneys, and is undergoing clinical evaluation at present.

The alternative to hypothermic perfusion is normothermic perfusion using a red-cell-based perfusate for the duration of the preservation period [13]. This approach avoids sustained periods of CI but does not circumvent the WI before the organ is placed on the machine or during implantation. Alternatively, a brief period of normothermic perfusion prior to implantation has been suggested to be of benefit [96], where the organ is perfused in the absence of some of the mediators of reperfusion injury such as platelets, neutrophils, and complement. The rationale is that this approach reduces the detrimental impact of some of the effector arm of the innate response to the inflammatory mediators that are released upon reperfusion. Not only may a period of machine perfusion enhance organ survival, it also allows assessment of function prior to implantation [97,98]. This may allow, for example, the identification of organs that are likely to have endured irrecoverable ischemic injury during the donation process. A period of ex situ perfusion also affords the opportunity to intervene in the deleterious metabolic processes associated with mitochondrial injury (as discussed) with appropriate compounds, or to expose the organ to a controlled period of ischemic preconditioning [99] to minimize subsequent IR injury upon implantation.

Clinician’s Corner

The ability to preserve organ function during extracorporeal storage is essential for successful transplantation. To do this, ischemic injury should be minimized, not only during storage, but also during extraction and implantation. Ischemia results in a failure of mitochondrial oxidative phosphorylation to generate ATP, and in mitochondrial injury at reperfusion with the liberation of ROS and other proinflammatory factors.

Hypothermia reduces metabolic activity, which increases tolerance to ischemia, and cold storage preservation solutions mitigate the accumulation of metabolites such as lactate, but cannot prevent reperfusion injury and mitochondrial ROS generation with its sequelae. Continuous normothermic perfusion enables reduction in the duration of ischemia, and offers the opportunity to assess function and modify the reperfusion response, but entails a reperfusion injury when the organ is placed on the machine as well as during subsequent implantation. Hypothermic oxygenated perfusion reduces reperfusion injury by avoiding rapid oxidation and stimulation of RET as succinate is metabolized, thus avoiding generation of harmful ROS and other inflammatory mediators.

With the development of good static cold storage preservation solutions, research into organ preservation ceased to be a priority in favor of developing safe effective immunosuppressive regimens. The identification of new targets for intervention in moderating the effects of ischemia and reperfusion opens up a new era of research, and identifies new challenges.

Treatment of donors prior to organ retrieval poses ethical issues relating to consent, where there is debate as to whether such a trial requires consent, and if so from whom, the donor or the recipient(s). The success of transplantation also poses its own challenge, since the use of graft survival as an endpoint, where for kidneys and livers it stands at over 90% at 1 year, requires a large number of subjects to show a benefit, the use of surrogate clinical or metabolic endpoints of IR injury will therefore be required.
Concluding Remarks

Hopefully, this review provides some new insights into understanding the limitations of current practice and point us towards new interventions and practices to both improve the outcome for patients and to increase the pool of available organs (see Clinician’s Corner). Our view is that now is a good time to look at transplantation from a mitochondrial and metabolic perspective. This perspective, which has been relatively neglected over the past few decades should comple- ment the progress in transplantation that has come from our understanding of how to counter graft rejection and to improve surgical techniques. Better understanding of the mitochondrial and metabolic contributions to transplantation are likely to lead to new pharmacological interventions and changes in clinical practice that could be applied at all stages of transplantation to improve outcomes. It will be interesting to see how these changes can be applied in conjunction with new developments in the ex vivo perfusion of organs both to assess their function and to minimize damage (see Outstanding Questions).

Acknowledgments

The research was funded by the National Institute for Health Research Blood and Transplant Research Unit (NIHR-BTRU) in Organ Donation and Transplantation at the University of Cambridge in collaboration with Newcastle University and in partnership with the National Health Service Blood and Transplant (NHSBT). The views expressed are those of the authors and not necessarily those of the NIHR, the Department of Health and Social Care or NHSBT. Work in the M.P.M laboratory is supported by an MRC grant (MR/P000320/1). We thank Hiran A. Prag for assistance with the National Health Service Blood and Transplant (NHSBT). The views expressed are those of the authors and not necessarily those of the NIHR, the Department of Health and Social Care or NHSBT. Work in the M.P.M laboratory is supported by an MRC grant (MR/P000320/1).

Disclaimer Statement

M.P.M. holds patents in preventing mitochondrial oxidative damage.

References


Outstanding Questions

Can the use of pharmacological interventions before and during organ retrieval and storage slow the degradation of adenine nucleotides?

Are there drugs or supplements that can be delivered to organs to prevent complete reduction of the NADH/NAD+ pool and thereby sustain glycolytic flux during ischemia?

Is it possible to decrease the accumulation of succinate during ischemia?

Can the glycogen levels in the organ within the donor be enhanced by dietary interventions prior to retrieval?

Slowing down ischemic metabolism is vital. Can this be achieved pharmacologically before ischemia, as is done by cardioplegia in the heart, or by more rapid cooling of the organs, or can cooling be accomplished while perfusing the organ, or even prior to the onset of ischemia by cooling the donor, in order to minimize the potentially damaging metabolic changes?

Upon reperfusion of the organ upon implantation within the recipient are there ways of preventing the ROS production and oxidative damage that underlies IR injury? For example, by blocking succinate oxidation or ROS production by complex I upon reperfusion, or by the use of mitochondria-targeted antioxidants?

Downstream of the IR injury that occurs upon implantation in the recipient, is it possible to block the induction of the MPTP with CsA, or to counteract the effects of the release of DAMPs from the mitochondria by drugs targeted to components of the innate immune system?
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