Strategies to Reduce Ischemia Reperfusion Injury in Vascularized Composite Allotransplantation of the Limb

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An important and often underinvestigated contributor to solid organ transplant rejection is ischemia reperfusion injury. This pathophysiological response releases damaging reactive oxygen species and cell stress signals that initiate inflammation, which has a critical role in priming the immune system for allorecognition. In time, this renders graft dysfunction and how this response is mediated in composite tissues remains unknown. Current protocols are drawn from solid organ transplantation with little scientific basis as to how this informs current hand transplantation practices. In addition to preservation flush and allograft cooling, machine perfusion is placing itself experimentally as a concept that could act to promote viability and increase the critical ischemic window, which is especially beneficial at a time of limited donors. With the increasing prevalence worldwide of hand transplantation, we review the potential contribution of ischemia reperfusion injury to hand allograft rejection including both current and experimental strategies. *(J Hand Surg Am. 2017;■(■):■−■. Copyright © 2017 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words Hand transplantation, ischemia reperfusion injury, vascularized composite allotransplantation, preservation.

THE U.S. NATIONAL DATABASE REPORT that amputation accounts for 3.6% of trauma presentations.[1] When the amputated part is irreparably damaged and not salvageable, vascularized composite allotransplantation (VCA) of the limb serves to provide "like for like" tissue for reconstruction. Over a million amputees in the United States alone could benefit; however, many obstacles exist to making this a provision available to all.[2]

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Received for publication February 20, 2017; accepted in revised form September 19, 2017.

K.R.A. is funded by the Engineering and Physical Sciences Research Council/Medical Research Council, British Society for Surgery of the Hand, and the Royal College of Surgeons of England. The rest of the authors declare that they have no relevant conflicts of interest.

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0363-5023/17/■ ■-0001$36.00/0
https://doi.org/10.1016/j.jhsa.2017.09.013

Success of transplantation has focused on the challenges faced by allograft rejection. A potential contributor to long-term success is the damage caused by ischemia reperfusion injury (IRI), an unavoidable series of events arising during reperfusion after an interval of cold static storage. This review aims to address current clinical and experimental preservation techniques that attempt to reduce this manifestation that is critical to solid organ transplant outcomes.

THE PROBLEM: ISCHEMIA REPERFUSION INJURY

Ischemia is inevitable following limb procurement. Composite tissues have different metabolic thresholds to the loss of blood supply, which differ depending on tissue type in both timing and intensity.[3] In particular, muscle is highly susceptible to ischemia and cell death.[4] The consequence of muscle and nerve dysfunction to a donor limb is profound because their physiological integrity is essential in providing physical function.

At a cellular level, ischemia causes hypoxic anaerobic glycolysis and oxygen consumption, which

depletes adenosine triphosphate (ATP) dysregulating ATP-dependent membrane ion exchangers. Potassium ions leak from hypoxic cells and intracellular sodium concentrations increase, resulting in cell swelling (Na^+/K^+-ATPase). Disruption of calcium-dependent exchangers increases cytosolic calcium (Ca^{2+}/Na^+-ATPase), and loss of this homeostasis leads to free fatty acid release and cell membrane disruption by activation of proteolytic enzymes. Collectively, this dysfunction manifests as cell apoptosis or necrosis.[5] When coupled with reperfusion, a localized microvascular and systemic response ensues that causes further tissue injury. Release of damaging reactive oxygen species (ROS), primarily from mitochondria, forms the hallmark of IRI. Endothelial injury upregulates the expression of bioactive agents (such as endothelin, thromboxane) and suppresses the release of nitric oxide (NO), which in turn increases vascular tone.[5] Together, in combination with free radical accumulation, endothelial swelling, and platelet activation, this can culminate in cessation of blood flow through the graft.[6]

Damaged tissues can activate the immune system by release of damage-associated molecular patterns, endogenous molecules that signal cellular injury. Their release upregulates endothelial adhesion molecules leading to the migration of leukocytes into the graft with activation of complement.[7] This localized inflammatory response is a barrier to the acquisition of tolerance. This explains why the magnitude and duration of IRI is proportional to posttransplant graft dysfunction and rejection (Fig. 1).

STRATEGIES TO MITIGATE ISCHEMIA

Protocols have adopted the need for a coordinated approach between solid organ and VCA retrieval teams. Currently, 1 of the key goals is to minimize the timing of retrieval and cold storage. Cold static storage forms the cornerstone of limb preservation in VCA, which encompasses an intravascular preservation flush followed by storage on ice until implantation.

Preservation flush

Preservation solution is infused after application of a high-arm tourniquet to minimize hemorrhage and preserve circulatory volume. In the event this is not feasible for logistic reasons, this is commenced after limb detachment. Preservation flush perfuses the microcirculation and protects tissue biochemical processes while concomitantly exuding stagnant blood. In conceptual terms, most preservation solutions aim to maintain electrolyte composition and counteract tissue edema and cell acidosis. The optimal preservation solution for the limb remains unknown, and to date, the most commonly reported solution is University of Wisconsin (UW), infused during the first human limb transplant in 1998 in Lyon, France.[8] The UW solution contains lactobionate and raffinose, metabolically inert substrates that maintain osmotic properties vital to ensure minimal fluid diffusion into the interstitium upon reperfusion. This is aided by hydroxyethyl starch, increasing oncotic pressure minimizing intracellular fluid shifts, further assisted by potassium acting on the (Na^+/K^+ATPase) exchanger. Addition of free radical scavengers such as glutathione, allopurinol or adenosine replenish cellular ATP. Experimentally, UW preserves muscle electrophysiology, morphology, and function.[9] However, the potential for vascular endothelial injury from potassium-induced vasoconstriction (required for UW cardioplegia of the donor heart) further highlights why limb-specific preservation solutions deserve further investigation.

Histidine-tryptophan-ketoglutarate, a low-viscosity cardioplegic solution, is the only other alternative solution used by a single hand transplant team. Experimentally, compared with UW, there is decreased inflammation and tissue damage primarily of skin and muscle.[10] Histidine-tryptophan-ketoglutarate has a low-potassium concentration that withdraws extracellular calcium and sodium to promote vasodilation. In view of the unique cellular and metabolic features of the allograft, a more scientific approach to selecting and developing preservation solutions is required.

Cooling

Cooling decreases cellular metabolism by 1.5- to 2-fold for every 10°C drop in temperature. This has been attributed to a reduction in degradation of intracellular enzymes essential for cell viability, which is accompanied by a reduction in consumption of ATP.[11] This is why preservation solutions are infused cold, a more effective method than topical cooling.[12] Low temperatures are maintained by placement of the allograft (sealed in a sterile bag) in iced water (4°C–6°C).[13]

Human cold ischemic time has ranged from 30 minutes to 13 hours (mean, 5.3 hours), with insufficient international hand registry data to define the upper limit. In a double hand transplant, Landin et al[14] reported muscle fibrosis in the limb with prolonged perioperative ischemia months following surgery. In 2 bilateral transplants, ischemic time