Ischemia/Reperfusion Injury in Kidney Transplantation: Mechanisms and Prevention

M. Kosieradzki and W. Rowiński

ABSTRACT
Ischemia has been an inevitable event accompanying kidney transplantation. Ischemic changes start with brain death, which is associated with severe hemodynamic disturbances: increasing intracranial pressure results in bradycardia and decreased cardiac output; the Cushing reflex causes tachycardia and increased blood pressure; and after a short period of stabilization, systemic vascular resistance declines with hypotension leading to cardiac arrest. Free radical-mediated injury releases proinflammatory cytokines and activates innate immunity. It has been suggested that all of these changes—the early innate response and the ischemic tissue damage—play roles in the development of adaptive responses, which in turn may lead to an acute font of kidney rejection. Hypothermic kidney storage of various durations before transplantation add to ischemic tissue damage. The final stage of ischemic injury occurs during reperfusion. Reperfusion injury, the effector phase of ischemic injury, develops hours or days after the initial insult. Repair and regeneration processes occur together with cellular apoptosis, autophagy, and necrosis; the fate of the organ depends on whether cell death or regeneration prevails. The whole process has been described as the ischemia-reperfusion (I-R) injury. It has a profound influence on not only the early but also the late function of a transplanted kidney. Prevention of I-R injury should be started before organ recovery by donor pretreatment. The organ shortage has become one of the most important factors limiting extension of deceased donor kidney transplantation worldwide. It has caused increasing use of suboptimal deceased donors (high risk, extended criteria [ECD], marginal donors) and uncontrolled non–heart-beating (NHBD) donors. Kidneys from such donors are exposed to much greater ischemic damage before recovery and show reduced chances for proper early as well as long-term function. Storage of kidneys, especially those recovered from ECD (or NHBD) donors, should use machine perfusion.

M OST OF THE DATA on mechanisms of ischemic injury in eukaryotic cells are derived from cardiac-myocyte studies. The heart can reveal many stages of ischemia-reperfusion injury, including stunning, hibernation, arrhythmias, and infarction. These stages are not as well defined in other tissues and organs. Ischemia of a transplant is somehow unique as complete cessation of the blood supply as is observed in a removed organ and hardly ever occurs in ischemic tissues—heart, kidneys, brain, liver, skeletal muscle—which are left in situ after arterial/portal flow occlusion. Herein, we discuss cellular mechanisms of ischemia-reperfusion injury of transplanted organs and methods to prevent this injury with available organ preservation methods.

CELLULAR AND MOLECULAR MECHANISMS OF ISCHEMIA
Deprivation of Oxygen
Cessation of arterial blood flow with immediate oxygen deprivation of cells (ie, hypoxia with accumulation of metabolic products) is defined as ischemic injury. A switch
to the anaerobic glucose metabolism pathway, most likely driven by a change in the oxeredox state with activation of glycolytic enzymes and their genes, occurs within minutes; its severity depends on metabolic demand of the tissue. Although a net synthesis of 36 molecules of adenosine triphosphate (ATP) for each glucose particle undergoing oxidative phosphorylation is not exactly true, anaerobic metabolism generates only a minimal amount of high-energy phosphates, which is definitely insufficient to meet the demands of aerobic tissues. In hypoxic muscle cells, glucose consumption was observed to be increased 12-fold. However, apart from provision of oxygen, ischemia also results in metabolic substrate starvation: fewer glycogen granules are observed in ischemic tissue. Although not the most important contributing factor, exhaustion of cellular of glycogen immediately arranges anaerobic glycolysis. More importantly, even if glycogen is still present but ATP has already been exhausted, phosphorylation of fructose-6-phosphate cannot occur, the glycolysis pathway is not “ignited.” The toxicity of metabolic products, which are not washed out or eliminated, increases in parallel with the osmolar load. Tissues accumulate inorganic phosphate, protons, creatine, and glycolysis products. Increased H⁺, lactates, and NADH inhibit glycolytic enzymes, namely glyceraldehyde phosphate dehydrogenase. In addition, ATP synthase now becomes a hydrolase, swiftly dephosphorylating ATP to adenosine diphosphate (ADP). Existing stores of phosphocreatine are lost within seconds of ischemia due to at least temporary dephosphorylating of ADP, which is then gradually catabolized to inorganic phosphate, adenosine, and inosine, which being permeable to the cellular membrane escape to the extracellular compartment. This event results in deprivation of substrate for the synthesis of high-energy phosphates even when there is subsequent restoration of blood flow. The decreased ATP phosphorylation potential determines cellular function at the end of the ischemia. Although ATP synthase activity is diminished after 1 hour of cold ischemia, it can be restored after reoxygenation. However, when ischemia lasts more than 24 hours and energy substrates are lost, synthase activity does not recover after reperfusion, leading to lethal cell injury.

Edema

High-energy phosphates are vital for most cellular functions: from maintaining homeostasis, signal integration transduction, cell proliferation and differentiation to execution of the apoptotic death cycle. Although membrane phosphatases act slowly during ischemia, ATP depletion inhibits Na⁺/K⁺ membrane phosphatase, thereby impairing the ability to maintain membrane potential and cell excitability, which require protection from gradient-driven K⁻ and Na⁺ ion trafficking. Sodium, however, enters the cytoplasm accompanied by large amount of water, producing edema, the degree of which is dependent on the extent and duration of ischemia.

An arrest of glycolysis due to NAD⁺-deficiency-driven, glyceraldehyde phosphate dehydrogenase inhibition results in accumulation of various intermediates—glucose, glucose-6 and -1-phosphates, α-glycerol phosphate—and products—lactate, NADH, H⁺. This greatly increases the osmolar load of ischemic cells. Also when ATP is gradually dephosphorylated to soluble adenosine and three particles of inorganic phosphate, the osmolarity rises greatly in the intracellular compartment. Hyperosmolarity also attracts water into the cell through simple diffusion, aquaporin, and chloride channels as well as the glucose transporter. Actually this mechanism, not Na⁺/K⁺ pump insufficiency, is believed to be responsible for ischemic edema. Ischemic injury to the phospholipid bilayer can also be of importance. Naturally, in a no-flow situations ischemia metabolites leak out, accumulating in extracellular space unless there is a balance between intra- and extracellular osmolarity and membrane permeability, which slows the progression of edema. The phenomenon becomes a major issue when the extracellular space is washed out with machine perfusion (MP) for graft preservation, particularly if the osmolarity of the perfusion solution was not augmented. The extracellular space can easily become hypotonic with accelerated edema formation.

Edema results in disruption of cellular membranes: not only the outer cellular membrane by opening of stretch-activated channels that counteract the volume increase, with dissipation of the semiconductance of the membrane, but also of endoplasmic reticulum (ER), Golgi apparatus, mitochondrial membranes, and cytoskeletal microtubules, which are ischemia time-dependent. The ER performs hundreds of biochemical reactions, which require a special compartmentalized cytoplasmic milieu based upon pH, ion concentrations, redox state, and catalysts. When this feature is lost, uniform conditions do not permit most of the activities. Edema of mitochondria elongates the path way of energy-rich compounds (phosphocreatine), which is needed to be covered from the inner mitochondrial membrane to the cytoplasm. Similarly, 3C-acids and glucose must travel the same distance. The mitochondrion loses coordination of the metabolic cycle and phosphorylation, leading to opening of the transition pore. Necrosis occurs when ATP stores are lost. When ATP is at least partially preserved, cytochrome c is released with caspase activation, resulting in cell death. When swollen mitochondria lose their cristae and show matrix densities, an irreversible phase of injury is set.

Acidosis is an immediate result of anaerobic glycolysis: as energy demand is high, reaction turnover must follow to generate sufficient ATP. However, NAD⁺, lost in the process, can only be regenerated with fermentation of pyruvate to lactic acid, which strongly acidifies the cytoplasm. In acidosis, NAD⁺ decomposition occurs with formation of glycolysis-inhibiting metabolites. To prevent excessive acidosis, phosphofructokinase, a key enzyme in the glycolysis pathway, is inhibited by the low pH. With no blood flow to remove the lactate, energy production is halted. A decreased pH is uniformly observed in ischemic tissues. Although it may be involved in protection on
reperfusion, 11 acidosis during coronary artery occlusion correlates well with tissue injury as measured with myoglobin release. 12 Noteworthy, acidosis impairs recovery of contractile and microvascular endothelial functions in hypothermia-arrested hearts not exposed to ischemia. 13 Opening of acid-sensing ion channels with calcium influx may be a universal pathophysiologic mechanism, though these channels have not been studied in liver ischemia. However, interstitial lactic acidosis in the liver strongly correlates with reperfusion injury as evidenced by 24-hour aspartate transferase (AST) levels >2000 IU/L. 14

Calcium Overload
Mitochondrial dysfunction is a critical event during ischemia, as it initiates both necrosis and apoptosis cascades during reperfusion. The organelle is both the site wherein noxious particles are produced and the preferred target of the injury. During ischemia, the Na+/Ca2+ antiporter stops pumping calcium out of the cell, since sodium accumulating within the cell cannot be removed by the ineffective Na/K-ATPases, leading the Na/Ca exchanger to start to work in the reverse direction. However, so-called intracellular calcium overload early in ischemia occurs mostly due to redistribution of calcium from ER stores. 15 Influx of extracellular calcium occurs only during prolonged ischemia and reperfusion. 16 Increased intracellular calcium activates calpain and causes translocation of Na/K ATP-hydrolase to the mitochondrial content of calcium. Equilibration of H+ inner mitochondrial membrane, which is almost impermeable to calcium necessary for mPT opening, thereby attenuating logical intervention, increases the trigger concentration of calcium overload early in ischemia occurs mostly due to redistribution of calcium from ER stores. 15 Influx of extracellular calcium occurs only during prolonged ischemia and reperfusion. 16 Increased intracellular calcium activates calpain and causes translocation of Na/K ATP-hydrolase to the mitochondrial content of calcium. Equilibration of H+ inner mitochondrial membrane, which is almost impermeable to calcium necessary for mPT opening, thereby attenuating logical intervention, increases the trigger concentration of calcium.

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deprivation need to respond quickly to energy demands, all enzymes needed for glycolysis are expressed constitutively. To boost the response, the CsrA/B system differentially regulates mRNA stability of genes involved in glycolysis, gluconeogenesis, and glycolysis. In contrast, redundant genes of the respiratory complex II are shut off. The protein content of enzymes involved in glycolysis may be quickly enhanced by binding of HIF1α to the genome and by de novo protein synthesis. Most glycolytic enzymes have HIF1α binding sites. Although there are varying opinions on gene expression during hypothermia, we believe that the increased mRNA results from RNA stabilization in the cold. There is little or no evidence that double-stranded DNA unfolding, nucleotide transport, and enzyme-dependent, energy-consuming mRNA synthesis occur in warm-blooded animals, since even in bacteria, a temperature downshift results in transcription arrest, which can only be restored with prolonged adaptation to cold.31

Various types of ischemia generate different free radicals: peroxynitrite anions, important in warm ischemia, do not take part in cold ischemic injury to the kidneys.32 In cardiac muscle, cold ischemic injury is rendered by superoxide and hydroxyl radicals as well as by hydrogen peroxide.33

The resistance of various cell populations to different types of ischemia is difficult to assess, since major morphological changes observable with light microscopy occur only after long periods of time; changes can be seen sooner when reperfusion with blood is allowed. Cardiac endothelial cells appear quite resistant to warm ischemia, as over 50% of them seemed viable after 12 hours of warm ischemia, as assessed with trypan blue exclusion, acetylcholine relaxation, and transmission electron microscopy.34 Major endothelial injury develops only when ischemia was followed by reperfusion. Thus, warm ischemic injury to cardiomyocytes is mostly due to free radicals and inhibited NOS from substrate deprivation, which are probably the primary noxious factors influencing postreperfusion coronary flow and contractile functions, phenomena that are not true for prolonged cold ischemia.35 Warm ischemia also renders prominent injury to hepatocytes, and reduction of the number of viable Kupffer cells.36 In contrast, cold ischemia followed by reperfusion causes marked changes in sinusoidal endothelial cells with early necrosis without influencing hepatocytes.37 In the kidney the primary target of injury by warm ischemia is proximal tubular cells. Although they can tolerate a short exposure, changes in transepithelial potential and cell-to-cell junctions appear early, increasing leakage and slowing active transport.38

Organs can tolerate prolonged cold ischemia or some warm ischemia without significant deterioration of function. However, when both factors act in the same tissue, they easily produce profound injury with marked cell death.39,40

BRAIN DEATH AND DONOR PRETREATMENT

Brain death results from a rapid increase in intracranial pressure due to hemorrhage or brain edema. Altered intracranial volume affects venous outflow, speeding up the increased pressure until brain structures are pushed toward to the foramen magnum, completely halting arterial blood flow. Ischemia of the pons drives the autonomic reactions of bradycardia, increased blood pressure, and respiratory irregularity. With distal progression of ischemia, necrosis of the vagal, cardiomotoric, and respiratory nuclei occurs with uncontrolled sympathetic reactions, unless there has been total sympathetic denervation due to death of the medulla oblongata. Thus, brain death causes two distinct hemodynamic phases: uncontrolled sympathetic activation with arterial blood pressures well above 200 mm Hg and tachyarrythmia, followed by a much longer hypotensive period resulting from sympathetic insufficiency, loss of vascular tonus, and decreased peripheral resistance.41 Early-phase catecholamine storm depends on the velocity of the intracranial pressure increase with heightened serum adrenaline concentrations by as much as 200- to 1000-fold.42 Increased hydrostatic pressure causes immediate tissue edema, which is often hemorrhagic.43

Necrosis of the hypothalamus and pituitary gland dissipates thermoregulation44 and hormonal homeostasis: serum thyroid hormones do not meet metabolic demand,45 diabetes insipidus is not controlled by vasopressin,46 production of cortisol is halted,47 and insulin release is inadequate.48 Free radicals and proteolytic enzymes are released, leading to slow, progressive, and inevitable death of cells and tissues via necrotic and apoptotic mechanisms.49 These changes result in deterioration of organ function in brain-dead donors, including impaired kidney function in both optimal and marginal donors,50 hence, even if the effect of cold ischemia was eliminated in an experimental setting. There is as significant influence of brain death on renal transplant function,51 including more frequent acute rejection episodes52 and decreased long-term survival.53,54 Brain death has been observed to increase tissue CD6855 CD4550 and CD8 infiltrates;59 ICAM, VCAM,60 and E-selectin expression61; as well as serum content of proinflammatory cytokines.58

To avoid or at least minimize the consequences of brain death, various experimental and clinical treatment methods have been applied to brain-dead donors. Early aggressive donor management with stabilization of donor hemodynamics has been shown to increase the number of donations and the organ yield.59,60 Interestingly the evidence suggests that treatment with small doses of catecholamines, especially dopamine, may improve renal graft function and survival.61,62 Despite doubts whether it has any benefit beyond that of aggressive fluid management with hemodynamic stabilization, the Papworth protocol of methylprednisolone bolus, vasopressin, triiodothyronine and insulin infusions and its numerous modifications seems to facilitate control of hemodynamic disturbances and increase the number of transplanted hearts.63
KIDNEY STORAGE

Kidney storage in hypothermia, which is necessary for logistical reasons, must maintain organ viability between recovery and transplantation. The importance of ensuring successful preservation of kidneys between retrieval and implantation has been long recognized. Two approaches have been developed to limit ischemic damage: cold static storage (CS) and machine pulsatile perfusion. Both technologies have continued to evolve since their early development. In 1967, successful organ perfusion preservation was introduced by Belzer. Two years later, Geoffrey Collins described a preservation solution with intracellular ionic contents, which allowed successful storage in simple hypothermia for up to 18 to 24 hours. Soon, Belzer and Southard developed a different type of medium for hypothermic storage, so-called University of Wisconsin (UW) solution, which has become a gold standard.

Simple static cold storage is the most widely used form of preservation in everyday clinical practice. It is simple and effective when a kidney from a standard deceased donor is stored for up to 24 hours. In last 10 years, a variety of solutions have been introduced into market for successful hypothermic storage of kidneys and other organs. However, another approach is needed for longer storage times and for preservation of kidneys recovered from extended criteria (or non–heart-beating) donors.

Hypothermic pulsatile perfusion for kidney storage before transplantation was introduced by Belzer more than 30 years ago. This preservation method was not widely used, because of the simplicity of cold storage, especially since the UW solution came to the market since it allowed successful preservation for up to 24 to 30 hours. In the last 10 years, increased interest in MP has grown due to the use of organs preserved after the ischemic phase, become edematous and leaky to proteins and small particles. As a source of both preserved and survival. In prospective and retrospective studies, Kwaitowski et al showed beneficial effects of MP on early and long-term graft survival. Furthermore, the incidences of chronic rejection and, interstitial fibrosis/tubular atrophy were significantly lower among MP-preserved kidneys.

A recent prospective, randomized Eurotransplant study that compared CS with MP for preservation documented the value of MP: this method decreased the incidence of DGF not only among standard, but also extended criteria, donors. MP also reduced the incidence, the duration, and the severity of DGF and ameliorated graft function of non–heart-beating donor kidneys after transplantation. As shown recently using long-term observation, MP was economically superior to CS.

Hypothermic MP not only facilitates longer storage, but also allows assessment of the extent of ischemic damage to the organ. MP preservation also allows an assessment of kidney quality before transplantation. Measurements of flow, resistance, lactate excretion, and alpha-GST in the perfusate yields possible predictors of whether the kidney will display immediate or delayed function after transplantation.

REPERFUSION INJURY

Reperfusion injury, as an effector phase of ischemic injury, develops during hours or days after the initial insult. Repair and regeneration processes occur together with cellular apoptosis, autophagy, and necrosis; the fate of an organ depends on whether cell death or regeneration prevails. As apoptosis needs energy and protein synthesis, it occurs mostly upon reperfusion. Cytochrome c release and caspase activation has been noted as early as 5 minutes after reperfusion, while it was virtually absent during prolonged ischemia of cardiomyocytes. When cytosolic calcium that had increased during ischemia returned to normal values, the cells were able to recover from injury. However, a progressive increase in cytosolic calcium marked an irreversible phase of injury. The rapid burst of free radicals shortly following reperfusion is a well-documented phenomenon. Most likely, discordant respiratory chain enzymes are the main source of radicals on reperfusion. Reduced ubiquinone reacts with free oxygen to form superoxide, which is metabolized to hydrogen peroxide by catalase, or extremely reactive hydroxyl radicals in the presence of ferrous or copper ions. Radical-mediated peroxidation of lipids in cellular and mitochondrial membranes occurs in double bonds, aromatic rings, and thiol groups, which are present in cardiolipin and other phospholipids. Free radicals probably trigger endothelial injury, since only after reperfusion do endothelial cells, which seem fairly well preserved after the ischemic phase, become edematous and leaky to proteins and small particles. As a source of both free radicals and potent radical-scavenging potential via cytochrome c, mitochondria seem to be a gatekeeper to cellular injury during reperfusion. The mitochondrial permeability transition pore which remains closed during ischemia due to inhibition by acidosis, opens upon reperfu-
significant increases after reperfusion. \cite{82,83} Neutrophils may cause direct cytotoxicity via generation of oxygen free radicals and release of cytokines. They control perivascular tissue edema, damage endothelial cells directly, and promote platelet aggregation. The extent of their infiltration is proportional to the magnitude of injury to the reperfused organ. Although reperfusion begins with relative hyperemia, often exceeding the normal blood flow rate, it is followed by much longer phase of diminished blood flow. Although mostly driven by decreased metabolic demand and extracellular edema, it depends in part on neutrophil and platelet accumulation within blood vessels, which attenuate flow and cause the “no-reflow” phenomenon; however, there is dispute concerning the negative role of flow reduction.

**PREVENTION OF REPERFUSION INJURY**

**Normothermic Reperfusion**

Parallel to the development of new preservation solutions for CS (Vasosol, T-Kyoto, SCOT, IGL), major progress in normothermic preservation or resuscitation of organs has been made. The Kootstra group presented spectacular results of resuscitation of kidneys that had been severely damaged by 2 hours of warm ischemia. When reperfused at near-normothermia with their own exsanguinous metabolic support medium, the kidneys were successfully rescued from lethal injury. \cite{85} These experiments have been lately followed by the Leicester group: renal blood flow, high energy stores, and renal function after warm ischemia and 16 hours of cold storage were nicely restored by 2 hours of normothermic resuscitation with donor blood. \cite{86} However, the most impressive clinical progress was reported with the TransMedics Organ Care System, which allows for extracorporeal normothermic preservation in a donor blood-based solution. The Leicester group showed the superiority of prolonged warm preservation of controlled and uncontrolled non–heart-beating kidneys. \cite{87} This technology also seems promising for preservation, viability assessment, and expansion of utilized non–heart-beating livers and cardiac allografts. \cite{88} Recently some groups have suggested beneficial effects of normothermic MP as a new approach in organ preservation and transplantation. \cite{90}

**Treatment of the Recipient**

To successfully prevent reperfusion injury, actions must be applied early; at the stages of donor hemodynamic disturbances, organ retrieval, and preservation. Some interventions, however, may prove productive when used during graft reperfusion in the recipient.

Although free radicals play an undoubted role in the pathology of the ischemia-reperfusion injury, administration of free radical scavengers at reperfusion seemed to yield questionable results until Land confirmed a significant late-term protection. \cite{91} Lately, pyruvate has been shown to directly detoxify peroxynitrite and \( \text{H}_2\text{O}_2 \), protecting against myocardial reperfusion injury. \cite{92}

As described above, cyclosporine inhibits permeability transition and, when administered on reperfusion, decreases creatine kinase release and infarct size in humans undergoing percutaneous coronary intervention for acute cardiac ischemia. \cite{93} This needs thorough evaluation in transplant models; it remains in contrast with the current policy to delay calcineurin inhibitor administration in renal ischemia-reperfusion injury.

Intermittent ischemia and reperfusion prior to prolonged ischemia, called ischemic preconditioning, has been shown to salvage the heart and some other tissues from prolonged damage, a similar phenomenon can be applied at the time of transplant implantation. If gradually elongated kidney reperfusion periods were interrelated with short-time ischemia, serum blood urea nitrogen and creatinine decreased faster than among non-postconditioned animals exposed to warm ischemia. The functional improvement was accompanied by more effective complex II mitochondrial respiration and lesser peroxide production and protein oxidation, \cite{94} as well as inhibition of apoptosis in the reperfused tissue. \cite{95} Although the phenomenon was confirmed in hearts, brains, and skin flaps, some doubts call for further studies. \cite{96}

Interestingly, postconditioning protection had been induced pharmacologically with administration of 3% \( \text{v/v} \) sevoflurane from the beginning of reperfusion of the heart, which decreased infarct size and mitochondrial injury, although hyperglycemia abolished this protection. \cite{97}

The role of hemeoxygenase 1, the enzyme that converts heme into biliverdin, carbon monoxide, and free Fe, has been extensively studied in protection from ischemia-reperfusion injury. Exposure of liver transplant recipient animals to inhaled CO decreased serum alanine transferase, hepatocyte necrosis, and neutrophil infiltrates in dose-dependent fash-
Ion. Noteworthy, HO-1 can be induced by simvastatin preconditioning. Interestingly, through its strong anti-inflammatory effect, nicotine has been shown to reduce tubular damage in experimental models of warm ischemia when administered before reperfusion. It prevented neutrophil infiltration, decreased CXC, KC, tumor necrosis factor-alpha, and high-mobility group box-1 (HMGB1) protein release. Less tubular apoptosis and proliferation were observed among nicotine-treated mice.

**ISCHEMIA-REPERFUSION AND IMMUNE INJURY**

Ischemic injury to an allograft significantly increases the risk of poor initial function, which in turn has been associated with an increased rate of acute rejection episodes. Although there was no satisfactory supporting hypothesis, many researchers thought the association between ischemia and graft immunogenicity to be reasonable. An elegant explanation came from Matzinger's injury theory to explain the links between tissue damage, innate immune responses differentiating "self" from "non-self" or "damaged-self," and communication of danger signals to adaptive immunity via Toll-like receptors and antigen-presenting cells. "Danger signals" or "alarmins" released during ischemia and reperfusion may include graft-derived DNA and RNA, oxidized proteins and lipids, HMGB1, uric acid, and calcium pyrophosphate crystals. Lately, HMGB1 has been shown to be actively secreted from at-risk cells via a free-radicals-dependent pathway during hepatocyte ischemia-reperfusion. This process requires intact TLR4 signaling and calcium-dependent kinases. TLR4 expression is increased in tubular epithelial cells following ischemia-reperfusion injuries. When TLR4 or its signaling pathway protein, MyD88, were absent, kidneys were protected from ischemia-reperfusion.

According to the injury theory, the less the initial insult, the smaller the agitation of adaptive immunity and the lower the chances for early and late responses to the allograft. Land immediately noticed how beautifully this theory explained his results of delayed kidney graft protection after administration of recombinant superoxide dismutase. Prolonged ischemia (>$15$ hours) indeed increases graft immunity; it was shown to be an independent risk factor for postrejection de novo production of high-titer anti-HLA class I panel-reactive antibodies. Circulating anti-donor antibodies as well as immunoglobulin and C4d deposits in renal glomeruli accompanied severe (72 hours of CS) ischemic injury occurring 7 days after engraftment. When ischemia was limited to 24 hours, no signs of damage or antibody-mediated immunity were present at day 7. Lately, we have shown that although MP is not an effective method to decrease immediate effects of ischemic injury, it significantly ameliorated the late histological injuries in renal allografts. Tubular atrophy and interstitial fibrosis (formerly known as CAN) were observed in 90% of biopsies from cold-stored and among 64% of machine-perfused kidneys with evidences of chronic rejection (according to Banff 2005 consensus) among 9% and 3% of renal allografts, respectively. In an exquisite study involving 600 protocol biopsies, Yilmaz et al linked the risk of chronic allograft changes, including interstitial inflammation and fibrosis, tubular atrophy, vascular wall and tubular basal membrane thickening, with the duration of cold ischemia.

**CLINICAL RELEVANCE OF ISCHEMIA-REPERFUSION INJURY**

Organ shortage is a universal problem. The waiting lists are growing in all countries with an increased gap between demand for and number of available organs. For this reason, extended criteria and non-heart-beating donors are widely used. These kidneys are more susceptible to ischemic damage, leading to DGF or primary nonfunction, as well as worse graft function and survival. Kidneys recovered from such donors should be stored using pulsatile perfusion, which allows better protection during preservation-related ischemia, as well as allows measurement of several parameters—flow, resistance, lactate excretion, alfa GST—which may be useful to assess the extent of ischemic injury.

**REFERENCES**


35. Hansen TN, Haworth RA, Southard JH: Warm and cold ischemia result in different mechanisms of injury to the coronary vasculature during reperfusion of rat hearts. Transplant Proc 32:15, 2000


