To what extent can limiting cold ischaemia/reperfusion injury prevent delayed graft function?

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Introduction

Renal injury may be caused by numerous factors, including ischaemia, toxins and autoimmune diseases. In recent years, chronic allograft nephropathy has become one of the main causes of late organ loss following transplantation. Several clinical and experimental studies have suggested that, in addition to alloantigen-dependent factors, several alloantigen-independent factors may be also involved in this process [1]. The most commonly accepted alloantigen-independent risk factors for chronic allograft nephropathy are cytomegalovirus infection, drug cytotoxicity, patient noncompliance, donor age, hyperlipidaemia and hypertension. These non-immunological factors certainly play an important part in the onset of chronic allograft nephropathy by directly damaging the transplanted kidney and also through their interaction with a variety of immune factors such as histo-incompatibility and acute rejection episodes. Non-immune renal injury of the kidney to be transplanted may also begin before the brain death of the donor and continue after transplantation [2].

Until recently, the impact of ischaemia/reperfusion injury (IRI), an antigen-independent event in the process of organ transplantation, on delayed graft function (DGF) and late allograft deterioration tended to be underestimated [3]. IRI is a complex sequence of events that involves depletion of cellular ATP, entry of calcium, sodium and water into cells, activation of endothelial cells and infiltration of neutrophils into ischaemic tissues [4]. The onset of non-specific inflammatory processes has been shown to initiate and modulate antigen-specific immunity [5]. The response to IRI closely resembles an immune response, and includes the upregulation of major histocompatibility complex (MHC) antigens [6] and adhesion molecules, and the production of cytokines and chemokines, leading to inflammatory infiltrates [7,8].

The exact role of IRI as an additional risk factor for DGF and kidney graft survival remains a matter of debate. Since the pig kidney seems to be the most attractive organ for xenotransplantation, we have developed an autotransplanted large pig kidney model to
investigate the influence of ischaemia/reperfusion and the conditions of cold storage on DGF [9]. Transgenic xenograft organs will certainly face a high risk of vascular rejection by humans, as a result of a process that involves the activation of endothelial cells. Because we feel that there is a need to optimize the conditions for preserving potential xenograft donor organs before proceeding to their clinical application, we have reviewed the different attempts to improve the conditions of tissue preservation and reduce the impact of IRI on renal function using the non-allogenic model of the autotransplanted pig kidney [9].

### Strategies for attenuating ischaemia/reperfusion injury

The main steps required to preserve the organ to be transplanted include flushing, cooling, and sometimes the use of drugs. Organs can be preserved either by gravity perfusion followed by cold storage or by continuous pulsatile, machine-driven perfusion. Cooling reduces the metabolic rate, minimizing enzymatic activity and energy substrate consumption. During ischaemia, the interruption of blood supply and the lack of oxygen lead to anaerobic metabolism, with a loss of energy substrates and the accumulation of hypoxanthine within the ischaemic cells. The depletion of energy substrates affects the membrane ionic ATPase pumps, tending to result in the accumulation of calcium, sodium and water in the cells, which in turn causes them to swell. Recent studies have shown also that the cold storage of human tubular cells causes a marked increase in free radicals [10]. The strategy commonly used to reduce ischaemic injury during cold storage is the rapid flushing and cooling of the organs to 4°C using a preservation solution to minimize enzymatic activity and energy substrate depletion. The preservation solutions (Euro-Collins (EC) solution and University of Wisconsin (UW) solution) commonly used contain impermeable ions to minimize cell swelling, and high concentrations of potassium to prevent intracellular potassium loss. Attempts have been made to add various pharmacological additives, such as energy substrates, colloids or antioxidants, to preservation solutions, but their supposed beneficial effects remain subject to caution. Both the EC and UW preservation solutions have their own limitations in preventing IRI, and, therefore, modifications of the ionic content of preservation solutions intended to reduce reperfusion injury have been proposed [11]. A modified UW solution with low potassium and high sodium contents has been shown to be at least as effective as the original UW solution in preserving organs [12]. The new high-sodium and low-potassium Celsior preservation solution (Instig-Sangstat, Lyon, France) has been shown to be effective in preserving hearts, but its efficiency for preserving livers and kidneys is still debated [13,14], and needs further investigation. Also, the addition of compounds such as nitric oxide donors [15], inhibitors of reactive oxygen species [16], 21-aminosteroids [17] and trifluoperazine [18] to preservation solutions has been shown to provide some protection against IRI. Pulsatile perfusion of the kidney recipient [19] and the use of pharmacological agents promoting renal blood flow [20] have also been shown to improve early graft function.

Reperfusion stimulates the conversion of hypoxanthine and xanthine to uric acid and the excessive production of reactive oxygen species. This may lead to lipid peroxidation of cell membranes and promote cell death through necrosis and apoptosis. Superoxide dismutase has also been shown to have a protective effect on renal warm ischaemia, to reduce the incidence of rejection and to increase long-term graft survival after cadaveric renal transplants [21].

### The consequences of cold storage on reperfused kidneys

The fact that transplants from living donors have better long-term survival than those from cadavers suggests that IRI could be implicated in the pathogenesis of chronic transplant dysfunction [22]. However, the implication of cold ischaemia in DGF still remains a matter of debate. Experimental findings have suggested that cold ischaemia can rapidly affect the cytoarchitecture and function of tubular epithelial cells. Breton and Brown [23] have shown that cold-preservation of tissues affects the microtubule network and the localization of some apical membrane proteins, such as the highly glycosylated protein gp 330 (megalin) in the proximal tubule, or the vasopressin-regulated water channel protein aquaporin-2 (AQP2) in the collecting duct. Also, it has been shown that the membrane-associated cytoskeletal proteins, ankyrin and fodrin, and the basolaterally-located Na-K-ATPase pumps can initially be redistributed through the cytoplasm in proximal tubule cells of transplanted human kidneys [24]. Tullius et al. [25] have shown that long-term rat kidney isografts exhibit morphological changes that mirror those of chronic allograft renal dysfunction. Furthermore, human kidney grafts exposed to prolonged cold ischaemia more often exhibit early acute rejection episodes than those exposed to minimal ischaemia [26].

In an attempt to elucidate and perhaps even subsequently prevent the harmful impact of IRI on DGF, we have developed a model of autotransplantation using large pig kidneys [9]. Using this model, we showed that the development of inflammatory injury was closely correlated to the conditions of cold preservation, and that the addition of the anti-ischaemic drug, trimetazidine (TMZ, 1-[2,3,4-trimethoxy-benzyl] piperazine, 2 HCl), which prevented renal injury in the isolated perfused pig kidney exposed to prolonged cold ischaemia [27], also had significant beneficial effects on the renal function of autotransplanted pig
kidneys [9,28]. TMZ has been registered since 1978 and marketed in a number of countries as a safe drug able to prevent cellular ischaemia without producing adverse haemodynamic effects. Although the clinical efficacy of TMZ has been demonstrated in several double-blind trials, and its anti-anginal effect shown to be equivalent to that of propranolol, the molecular mechanism of its anti-ischaemic effects is not yet fully understood. TMZ has been shown to improve energy metabolism and ATP synthesis in various models of myocardial and liver ischaemia [29–31]. Several studies have investigated the protective effect of TMZ and have shown that this anti-ischaemic agent is able to inhibit the oxidation of palmitoyl carnitine and to reduce the deleterious rise in acyl carnitine levels induced by ischaemia [32].

Is there a link between ischaemia/reperfusion injury and the onset of interstitial fibrosis?

Fibrosis is the final common pathway for nearly all forms of disease that progress towards end-stage organ failure. Concomitantly with inflammation and injury, a number of factors are released by infiltrating cells and resident cells, and these can stimulate other cells in the inflamed tissue to produce basement membrane and extracellular matrix molecules, such as type I, III and IV collagens, fibronectins and proteoglycans. Using the model of autotransplanted pig kidney, we have shown that the cells recruited into an inflamed interstitium in the early phase of fibrosis consist mainly of CD4+ lymphocytes and macrophages and that the number of infiltrating monocytes/macrophages and CD4+ and CD8+ lymphocytes depends mainly on the initial conditions of cold preservation [9,28]. Also, we tested the effects of polyethylene glycol (PEG), particularly PEG 20 M, used as a colloid during cold preservation, on renal function and on the occurrence of interstitial fibrosis in the autotransplanted pig kidney model. This colloid can form a reversible complex with lipids in cell membranes and prevent osmotic swelling [33]. We and others have demonstrated that PEG reduces lipid peroxidation [34] and modifies or reduces immunogenicity when combined with normally immunogenic antigens [35–37]. These findings led us to try to find out whether adding PEG to the preservation solution could be sufficient to prevent IRI, cell infiltration and fibrosis [38]. In this preliminary study, we have shown that adding PEG to a simplified high-potassium perfusion solution both improves renal function and significantly reduces early cellular and interstitial infiltrates in retransplanted pig kidneys, as compared to retransplanted kidneys cold-flushed with conventional EC and UW solutions [38]. These promising findings call for further studies to assess the long-term capacity of PEG alone and its association with anti-ischaemic drugs in preventing chronic transplant nephropathy.

Conclusions and perspectives

Chronic transplant nephropathy is a multifactorial process. It is the main cause of late graft loss. Morphologically, it is characterized by interstitial fibrosis, tubular atrophy and glomerular sclerosis. Therapeutic strategies have been developed primarily to prevent and/or treat acute rejection resulting from the elimination, weakening, or poisoning of T lymphocytes. Although the early acute rejection rate has been reduced to below 20%, and the 1-year renal allograft survival increased to well over 80%, this improved early graft survival has not led to any significant improvement in long-term graft outcome.

Chronic transplant nephropathy is caused by both alloreactive and non-alloreactive factors. Cold ischaemia may lead to both alloantigen-independent lesions and allogenic-mediated injury. The inflammation due to IRI, which is largely independent of immunological processes, is clearly one of the main early deleterious events that promote chronic renal dysfunction and graft loss. More attention should be devoted to preventing the initial injury caused by IRI. This should allow optimized organ preservation before transplantation.

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