

Role of ischemia-reperfusion in oxidative stress-mediated injury during kidney transplantation

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Abstract

Renal transplant (RT) is the definitive treatment for end-stage renal disease, which is known as a high prevalence pathology with strong economic repercussion both for patients and health systems. Solid organ transplantation is a classically described clinical setting in which massive amounts of reactive oxygen species (ROS) are produced due to ischaemia-reperfusion, thus becoming an essential pathophysiological element involved in delayed graft function in the context of RT. Nevertheless, no clinical protocol yet exists to counteract the damage mediated by ROS intensively produced throughout the transplant process. The available evidence shows a number of successful experiences in the use of antioxidant supplementation and reinforcement over other oxidative stress-related pathologies. This article addresses the pathophysiological role of oxidative stress in RT and its known consequences in function and structure of the allograft, with the objective of gathering consistent information that demonstrates the central role of oxidative stress in this pathology, and to consider it as a possible therapeutic approach in the future.

Introduction

Worldwide, chronic kidney disease (CKD) is a highly prevalent pathology [1]. The main underlying diseases associated with CKD are hypertension and type 2 diabetes mellitus [2], with a prevalence of 27,6% and 12,3% respectively, according to data from the 2016-2017 Chilean National Health Survey [3]. National prevalence for this disease has not yet been determined in Chile, but the estimated global prevalence for CKD is 10% [4] and currently 1260 patients per million inhabitants are undergoing chronic hemodialysis, compared to 12.7 patients per million in 1980, according to data from the dialysis registry of the Chilean Nephrology Society [5]. According to estimates from the 2015 Global Burden of Disease study, approximately 1.2 million deaths are attributable to CKD [6], making it plausible to state that kidney disease could contribute more mortality than the main four target diseases defined by the WHO Global Action Plan for the Prevention and Control of Non-Communicable Diseases [7]. Reports from US Renal Data System show that in 2014 there were 120,688 new cases of end-stage renal disease (ESRD, defined as a glomerular filtration rate of less than 15 ml/min/1.73 m²) and 678,383 people were treated for this disease, indicating a rise in comparison to 2013 [8]. In addition, ESRD has a high economic impact on healthcare systems: in the US, Medicare cost of this disease has grown 57% from 1999 to 2004, representing 6,7% of the total medical expenditures.

CKD ultimately results in kidney failure, which requires treatment by renal replacement therapy (RRT): peritoneal dialysis, hemodialysis or renal transplantation (RT). Amongst them, RT is the gold-standard treatment for patients with ESRD, being the most cost-effective measure compared to other RRT options: US estimates suggest that annual Medicare cost of RT is lower than hemodialysis and peritoneal dialysis [9,10]. Cardiovascular event-related mortality is 10 to 30 times higher in patients undergoing hemodialysis than general population [11]. Despite this risk being lower in RT recipients compared to hemodialysis patients, it is still 2 times higher than in healthy patients [12].

Ischaemia-reperfusion (IR) is an inevitable condition during RT, with several studies reporting an association of IR during RT with an impairment in both short and long-term graft survival [13,14]. Despite the above, no clinical protocol yet exists to counteract the damage produced by IR throughout the transplant process.

Role of ischaemia/reperfusion in renal transplantation: pathophysiological bases

Ischaemia-reperfusion injury (IRI) is defined as the damage produced in a tissue subsequently to blood flow restoration (reperfusion), after being previously subjected to limitation of blood flow (ischaemia) for a period of time extended enough to generate hypoxic damage.

Clamping and section of the renal artery during kidney procurement surgery mark the start of renal ischaemia, to be later reperfused after arterial anastomosis in the recipient. Both ischaemia and reperfusion have been demonstrated to be sources of production of reactive oxygen species (ROS), and ultimately producing oxidative stress-mediated damage in the transplanted organ.

Tissue hypoxia generated during ischaemia induces a metabolic shift in the cell from aerobic to anaerobic pathways, which entails a decrease in the generation of adenosine triphosphate (ATP) and an increase in lactate concentrations, leading to intracellular acidosis [15]. ATP depletion causes an electrolytic alteration consisting of an

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increase of Na⁺ and water influx, and an intracellular Ca²⁺ overload [16]. Furthermore, hypoxia reduces the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and inhibits the expression of cytochrome c oxidase, increasing the production of ROS following an oxygen concentration increase (e.g. during reperfusion) [17,18]. The previously described events produce uncoupling of the mitochondrial respiratory chain and weakening of the antioxidant system, allowing a ROS burst during reperfusion, which causes oxidative damage to biomolecules including proteins, lipids and deoxyribonucleic acid (DNA), ending in processes of apoptosis and cell death [15]. In addition, evidence suggests that NADPH oxidase (an enzyme complex that catalyzes the production of a superoxide free radical by transferring one electron from NADPH to oxygen) is activated during the IRI process, having a potential role in the pathophysiology of renal damage in this particular context [19] (Figure 1).

Clinical correlation: ischaemia-reperfusion injury and delayed graft function

The most frequent post-RT complications are acute kidney injury and acute and chronic graft rejection. The development of these phenomena is multifactorial, including IRI and host/recipient histocompatibility profiles[15,20]. Therefore, many functional parameters in the graft are useful to assess the risk of developing these complications, but one of them has significant clinical relevance because of its frequent incidence in RT and its correlation with long and short-term allograft outcomes [15,21,22]: delayed graft function (DGF), which can be defined as the requirement of at least one dialysis session during the first week post-transplantation [23].

There are different risk factors involved in DGF development, a main one being IRI [24,25]. Moreover, several studies confirm the correlation

between oxidative stress and DGF development, for example, by linking glutathione S-transferase (antioxidant enzyme) polymorphism GSTM1 and GSTT1 in donors and recipients, to DGF and OS parameters in RT recipients. The results showed that lipoperoxidation, an OS parameter, was significantly higher in patients with DGF and also the frequency of GSTM1 null was significantly higher in patients who developed DGF [26]. Another study Mandegary, *et al.* investigated the association between p22^{phox} (C242T), a polymorphic subunit of NADPH-oxidase, which has a critical role in the activation and stabilization of this pro-oxidant enzyme involved in the production of superoxide, triggering inflammatory injuries to the kidney. The results showed a significant association between p22^{phox} C242T polymorphism in the recipient and DGF occurrence [27].

Also, published data La Manna, *et al.* demonstrated the correlation between low levels of DNA oxidation and apoptosis at 6 months post-RT, with an improved function recovery in kidney allografts, supporting the main role of oxidative stress in graft outcomes [28]. Furthermore, it has been established that malondialdehyde (MDA) levels, a lipoperoxidation and oxidative stress marker, were consistently higher in RT recipient patients that developed DGF compared to those who did not. Also, MDA levels were lower in patients with a better renal function over time. First-day MDA measurements after RT were proven useful as an early marker for DGF [29].

Evidence regarding antioxidant supplementation in ischaemia/reperfusion models

Given the proven association between IRI, oxidative stress and graft outcomes, several authors have investigated the use of antioxidant therapy in RT.

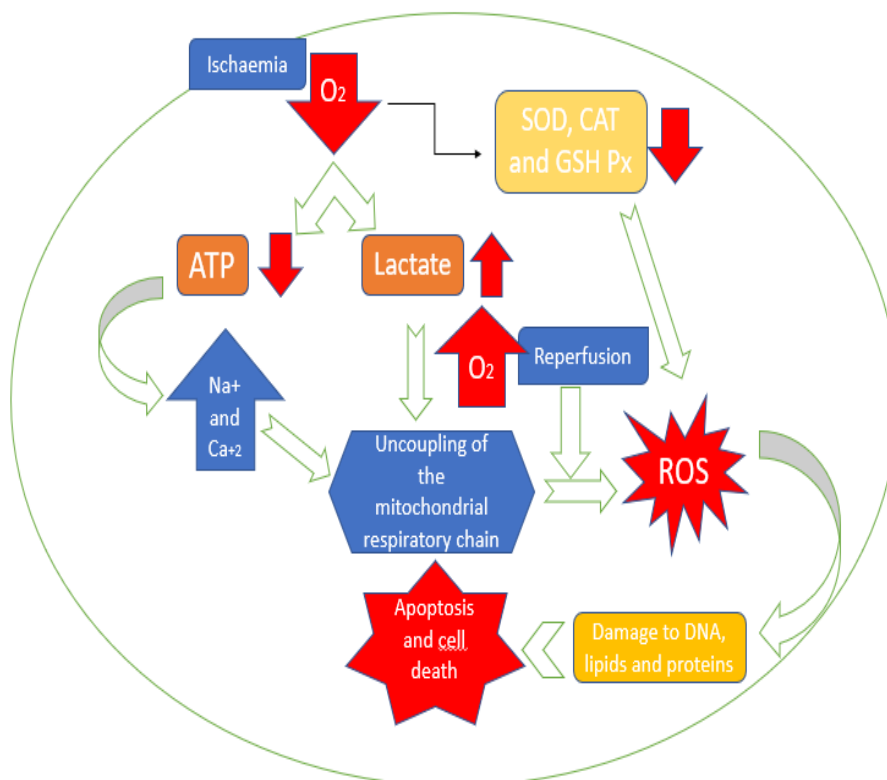


Figure 1. Role of ischemia-reperfusion in the generation of damage mediated by oxidative stress.

Long, *et al.* studied the attenuation of IRI in mice kidney by preconditioning with oleanolic acid, a natural triterpenoid present in various food products, like vegetable oils, with a proven antioxidant activity [30,31]. The protocol consisted in the distribution of rats into 3 main groups; a sham group, which received sham surgery, an ischaemia-reperfusion group, and an ischaemia-reperfusion group that received an injection of oleanolic acid for 15 consecutive days prior to the induction of renal ischaemia-reperfusion. The renal function assessment was measured by determination of blood urea nitrogen, creatinine, kidney injury molecule-1 (KIM-1) and others. Besides, antioxidant activity was assessed by MDA levels, SOD, CAT and GSH-Px activity. The results demonstrated that preconditioning by oleanolic acid was able to prevent IR-induced renal injury, as evidenced by decreased serum levels of blood urea nitrogen, creatinine and lactate dehydrogenase, and renal levels of KIM-1, compared with the I/R group. Furthermore, said preconditioning exhibited antioxidant effects in IR-oleanolic acid, as reflected by decreased MDA levels, increased SOD, CAT and GSH-Px activities, and increased GSH content compared with the IR rats [32]. Another study of natural antioxidants supplementation is the use of leutoline (Liu, *et al.*), a flavonoid obtained from a variety of plants like carrot, peppers and olive oil that has shown potent antioxidant properties [33]. Similarly, to the previously described, this study split rats into 3 groups, a sham group, an ischaemia-reperfusion group and an ischaemia-reperfusion group supplemented with leutoline. The results reported a significant enhance of renal function biomarkers (i.e. blood urea nitrogen and creatinine levels) and antioxidant enzyme function (e.g. CAT, SOD and GSH-Px) in the supplemented group compared to the ischaemia-reperfusion group [34].

Other studies were focused on the use of non-polyphenolic substances, such as Cusmano, *et al.* who evaluated the effect of atorvastatin (ATOR) and N-acetylcysteine (NAC) in the prevention of IRI on a murine model. Both substances possess anti-inflammatory, antiapoptotic and antioxidant properties. Mice were divided into 4 groups: control, NAC, ATOR and NAC+ATOR. Myeloperoxidase (MPO), SOD, CAT and GSH-Px levels were measured in renal tissue, plus the assessment of microstructural alterations (glomerulopathy, interstitial infiltrate, tubular anomalies and vasculopathy). GSH-Px activity levels were significantly higher and MPO activity levels were significantly lower in the three treated groups compared to control group, in addition to a lower ischaemic tubular injury index in NAC+ATOR group compared to placebo [35]. Another research analyzed the therapeutic effects of NAC in deceased donor RT, related to oxidative stress (Danilovic, *et al.*). Variables measured included creatininemia, GFR estimated by MDRD and Cockcroft-Gault methods (eGFR), the incidence of DGF and the analysis of reactive substances of thiobarbituric acid (TBARS), an oxidative stress biomarker. Two groups were established: NAC and placebo. NAC treated group showed lower creatininemia and higher GFR estimation during the first 90 days post-transplantation and a year after RT, compared to control group. Furthermore, rate DGF and TBARS levels were significantly lower in NAC treated group compared to placebo [36].

Conclusions

The available evidence allows us to conclude that during RT, inevitable damage is produced by ischaemia-reperfusion, which involves a decrease in antioxidant enzymes activity and enhanced ROS production resulting in cell death and the incidence of negative graft outcomes. Furthermore, there is a correlation between IRI, oxidative stress parameters and DGF, evidenced both in clinical and pre-clinical

antioxidant supplementation models. Nevertheless, more solid studies are necessary to prove the efficacy of antioxidant supplementation in RT and to perfect a therapeutic strategy. Finally, an approach to RT management and care under the oxidative stress paradigm is an exciting, new path that has yet to be fully understood, but could potentially offer novel, safe and cost-effective treatments aimed to obtain optimized graft outcomes.

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