Ischemia/reperfusion injury : a metabolic meltdown

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I

GENERAL INTRODUCTION

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PROBLEM DEFINITION

Kidney transplantation is currently the only curative option in end-stage renal disease, with some 80,000 people globally receiving a kidney transplant each year. Thus the success rate of transplantation surgery is critical, but graft failure is often a major problem. The exact mechanisms that lead to graft failure for both the short- and longer-term are unknown, however the damage resulting from the period of graft ischemia and reperfusion (I/R) is a major contributor.
I/R injury following kidney transplantation leads to delayed graft function (DGF)[1] in the short term. DGF is defined as the situation in which the recipient of the kidney transplant is dialysis dependent for the first week(s) after transplantation.[2] Besides the negative short-term effects of DGF (increased morbidity for the graft recipient and higher costs of prolonged hospitalization), DGF also impairs the long-term transplantation outcome. For example, incident DGF increases the risk of chronic allograft nephropathy and impairs long-term graft survival.[1,3,4]
Donor organ shortages force the use of so-called 'expanded criteria donor' organs in kidney transplantation. These organs are either donated after a cardiovascular accident or donated by an older donor (›60 yrs. or 50-59 yrs. with comorbidities).[5] Use of these grafts leads to an higher incidence of DGF and the associated negative consequences.[1,6] Over the past decades millions of dollars have been spent on research focused on finding interventions to limit I/R injury. However, promising results from preclinical studies could not be translated to the clinical setting - this translational gap is yet to be bridged.

OBJECTIVE OF THIS THESIS

This thesis aims to increase the knowledge concerning (human) pathophysiological mechanisms of I/R injury. Unravelling the underlying mechanisms of I/R Injury is the first step to identifying targets for limiting or preventing injury and thereby improving transplantation success rates. To achieve this, this thesis describes an unbiased, genome-broad, metabolome-broad and epidemiological approach to the mechanisms of I/R injury in the clinical setting of kidney transplantation.

BACKGROUND

For decades, transplantations have been an area of major interest and research efforts have resulted in significant developments and new discoveries. Since the first successful living-related donor kidney transplantation in 1954, significant improvements have been achieved in both patient and graft survival. One of the first major breakthroughs was the discovery of the human leukocyte antigen (HLA) system - a gene complex encoding the major histocompatibility complex proteins. HLA typing instigated international HLA matching programs for donor organs.[7]

Later, the induction and maintenance immunosuppression regiments were introduced to reduce the risk of acute rejection.[8,9] Another important advance was the development and optimization of graft preservation fluid (i.e. UW, HTK).[10,11] However despite all these successes, incident DGF following kidney transplantation remains a major problem.

I/R INJURY IN KIDNEY TRANSPLANTATION

Several promising preclinical studies have been published on limiting I/R injury in kidney transplantation. The main objective has been suppressing the inflammatory response or the adaptation of the immune system of the recipient.[6] Much attention has been given to ischemic pre- and post-conditioning. It is hypothesized that pre-/post-conditioning activates anti-inflammatory, neuronal and humoral signalling pathways – potentially protecting the reperfused graft.[12] Other examples of widely studied anti-inflammatory interventions are the administration of vitamins[6] / antioxidants[13] (i.e. n-acetylcysteine[14]) and volatile anaesthetics[15].

Generally these interventions showed positive results in preclinical models of kidney transplantation, but evidence of clinical effectiveness is still lacking.[16,17] (i.e. ischemic pre- and post-conditioning[18–21]; anaesthetic preconditioning[22]; vitamins/antioxidants[23] (N-acetylcysteine[24]) and EPO administration[25]). It appears that the translation of interventions reducing I/R injury from bench to bedside is limited.[26–29]

In addition to interventions that are administrated after reperfusion, many studies focus on the preservation phase.[30,31] Currently, static cold storage and machine perfusion are the two main options for donor kidney preservation. Static cold storage has proven to be a consistent, reliable method; however as the composition of preservation fluids has not altered over the last decennium further innovations are not expected.[32] Machine perfusion has rapidly developed over the past years and recently a meta-analysis concluded that short-term outcomes of hypothermic machine perfusion are superior to those of static cold storage. However long-term outcomes are less clear[33] and results of new machine (varying the perfusion fluid, hypo- versus normothermic and oxygenated protocols) must be awaited.[33–36]

GENERAL MECHANISMS OF I/R INJURY

The mechanism of I/R injury is multifactorial and complex, comprising a metabolic imbalance and microvascular dysfunction during ischemia which leads to activation of innate and adaptive immune responses and cell death programs after reperfusion.[37] Several pathways have been suggested as culprit mechanisms including: vascular leakage (endothelial activation), no reflow phenomenon (endothelial/thrombogenic), complement activation, cell death programs

(apoptosis, necrosis, autophagy) and innate and adaptive immune activation.[37]
With regards to the translational challenges, the NIH Consortium for "preclinicAl AssESsment of CARdioprotective therapies" (CAESAR) wrote:
'*For 40 years, the National Heart, Lung, and Blood Institute (NHLBI) has invested enormous resources (at least several hundred million dollars) in preclinical studies aimed at developing infarct-sparing therapies, and several hundred (if not thousands) therapies have been claimed to limit infarct size in preclinical models. Unfortunately, due largely to methodological problems, this enormous investment has not produced any notable clinical application, and no cardioprotective therapy is currently available for clinical use.*'[17]
Consistent with the above, almost all therapies aiming to minimize I/R injury deducted from animal models lack proof in (sufficiently powered) randomized clinical trials (RCTs)[16,38,39] highlighting the lack of understanding of the pathophysiologic mechanism of I/R injury in humans.
Previous research by de Vries et al. on I/R injury in human kidney transplantation describes a systematic re-evaluation of the presumed mechanisms. De Vries' thesis concludes that mechanisms commonly implicated in experimental I/R injury do not play a key role in clinical kidney transplantation.[26–28,40] For example complement, thrombocyte and endothelial activation were all absent in I/R injury following kidney transplantation.
Cytokine release (IL-6, IL-9) was associated with I/R injury, but inhibition of these cytokines in an experimental model resulted in increased renal damage[28] implying a protective role for inflammatory processes in I/R injury. Remarkably the release of ROS (radical oxygen species)-mediated damage biomarkers after reperfusion could also not be demonstrated. This finding was rather controversial as in the field of I/R injury ROS generation is considered to be the major driver of I/R injury. The common concept of ROS being the major driver has led to extensive studies on antioxidants aiming to limit I/R injury. Despite finding antioxidants protection in experimental models, no antioxidant has proved itself to be effective in treating or preventing clinical I/R injury.[23,41] This supports our theory that the role of ROS is less important than commonly thought.

CONTENT OF THIS THESIS

This thesis contributes to bridging the translational gap from bench to bedside, laying a foundation for effective new treatment measures for I/R injury. Key questions addressed include:

> Why do promising preclinical findings not translate to clinical settings?
> Why did almost all clinical trials aiming to limit DGF (thus I/R injury) result in negative results?

Which pathophysiological mechanisms could underlie DGF after human kidney transplantation?
Which interventions could hypothetically decrease the chance of DGF after human kidney transplantation?
Which variables (donor / transplant procedure) affect the long-term outcome of kidney transplantation and does this have implications for the future donorpool?

In order to answer the questions above kidney transplantation was used to examine human I/R injury, as the I/R injured organ is accessible and the setting potentially offers consistency for the replication of experiments.

MODEL

During kidney transplantation, both initiation of ischemia and reperfusion are planned. The duration of ischemia is monitored and can be used as a variable. This unique situation allows kidney transplantation to be seen as "an experiment by nature". The studies summarized in this thesis are more difficult to perform in 'spontaneous' I/R like myocardial infarction or stroke. This is illustrated by the arteriovenous sampling method that was performed: the artery and renal vein were cannulated during the transplantation procedure and after initiation of reperfusion samples were taken on fixed moments.(Fig.1) Another benefit of transplantation compared to myocardial infarction and stroke is that transplantation always assures whole organ ischemia while infarct size differs between patients. Finally the transplantation setting advantageously provides a clearly quantifiable clinical readout of I/R injury: DGF.[1]

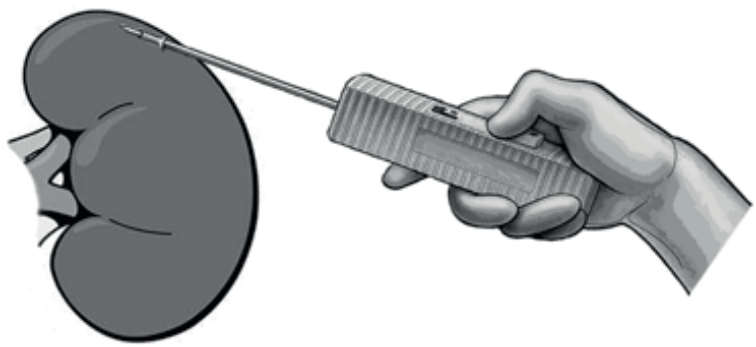

Figure 1A. Using kidney transplantation as a model of human ischemia/ reperfusion injury offers several benefits. During transplantation the kidney is accessible and biopsies can be taken during both ischemia and reperfusion.

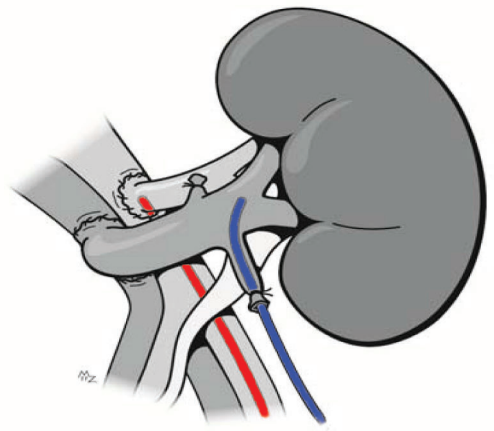

Figure 1B Kidney transplantation comprises a setting of whole organ ischemia, which enables arteriovenous measurements over the reperfused graft as a reproducible model.

OUTLINE OF THE THESIS

Following Chapter 1's general introduction to ischemia/reperfusion injury, the next 2 Chapters describe processes inconsistent with common I/R injury theories. Chapter 2 refutes the role of the hypoxanthine-XO (xanthine oxidase) axis in the development of DGF and discusses the role of ROS-mediated damage in I/R injury. Chapter 3 then examines whether succinate accumulation-induced ROS production drives I/R injury in human kidney transplantation. The Chapter pursues on interspecies differences in mitochondrial function and vulnerability against I/R, assessing the value of different animal models. Chapters 4 and 5 share new insights in human pathophysiology of I/R injury and identify targets that could limit DGF. Unbiased transcriptomic and metabolomic approaches describe processes and pathways underlying I/R injury. In both studies, grafts with a functional response upon ischemia and reperfusion (reference group of adequately recovering grafts) were compared with grafts that developed DGF after transplantation (i.e. I/R injury). Chapter 6 evaluates donor and transplantation procedure factors via a more epidemiological approach. Using the NOTR registry, long-term graft survival of grafts donated after brain death (DBD) and grafts donated after cardiac death (DCD) was compared. Analyses of the Leiden University Medical Center (LUMC) cohort were performed to evaluate functional graft recovery. Chapter 7 describes the role of mitochondrial pool recovery in advance of functional recovery after DGF. Chapter 8 elaborates on the role of mitochondrial (detoxifying) aldehyde dehydrogenase (ALDH) enzymes in I/R injury. Next the opportunity of simvastatine as protective pretreatment before I/R is described in

the exploratory trial in Chapter 9. Chapter 10 summarizes all the experimental and clinical studies performed on donor pretreatment in the context of kidney transplantation. To conclude, Chapter 11 summarizes the findings of this thesis and explains why unraveling the primordial triggers of I/R injury will provide focus in developing new strategies to mitigate clinical I/R injury.

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