Studying the short-term complications of kidney transplantation: from bed to bench

Kok, M.J.C. de

PART II

Studying the pathophysiology of metabolic failure in ischemia-reperfusion injury

Chapter 5

Clinical ischemia-reperfusion injury: driven by reductive rather than oxidative stress?

Michèle J.C. de Kok, Jonna R. Bloeme – ter Horst, Alexander F.M. Schaapherder,
Ian P.J. Alwayn, Rutger J. Ploeg, Jaap A. Bakker, Jan H.N. Lindeman

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Abstract

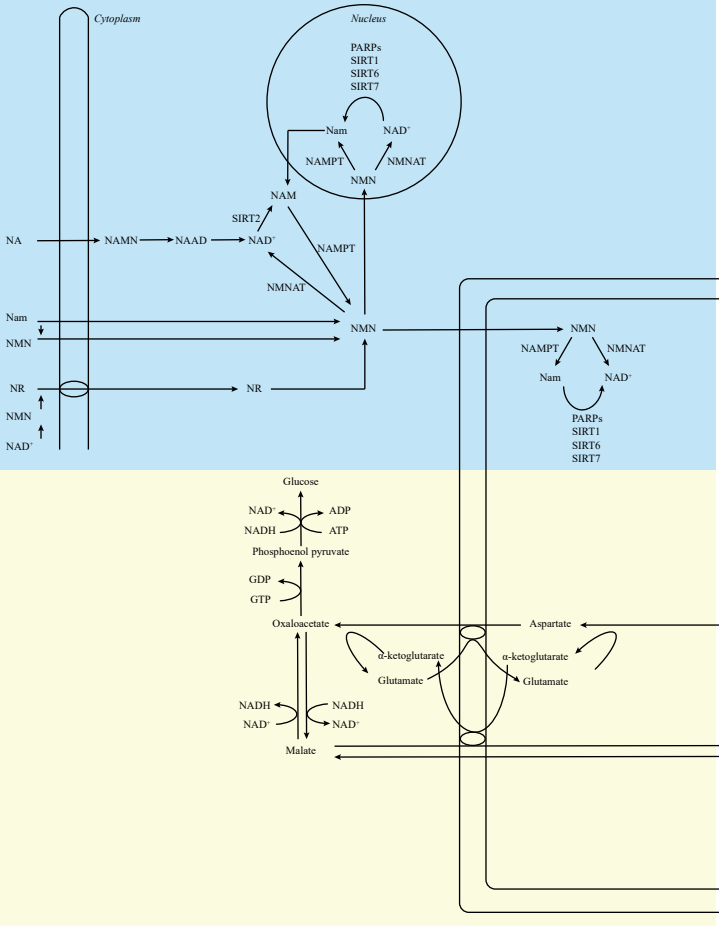
In clinical practice, ischemia-reperfusion injury (I/R injury) remains a major contributor to organ damage following transient hypoxic insults. Although numerous interventions have been suggested to be effective and to reduce I/R injury in preclinical models, none of these therapies have been successfully translated to the clinical setting. In the context of this 'translational gap' we have focused on recent clinical observations that have reported on a discriminatory metabolic signature of renal I/R injury, that in the clinical context manifested itself as delayed graft function after kidney transplantation. This signature points to the presence of a persistent postreperfusion metabolic paralysis, tricarboxylic acid cycle defects and a compensatory activation of catabolic routes. Against this background, the picture emerges that clinical I/R injury might be actually driven by reductive stress. In this perspective article, we have evaluated on the processes contributing to reductive stress in the context of I/R injury and try to provide better insight in potential clinical therapeutic strategies that may be helpful in restoring the redox balance.

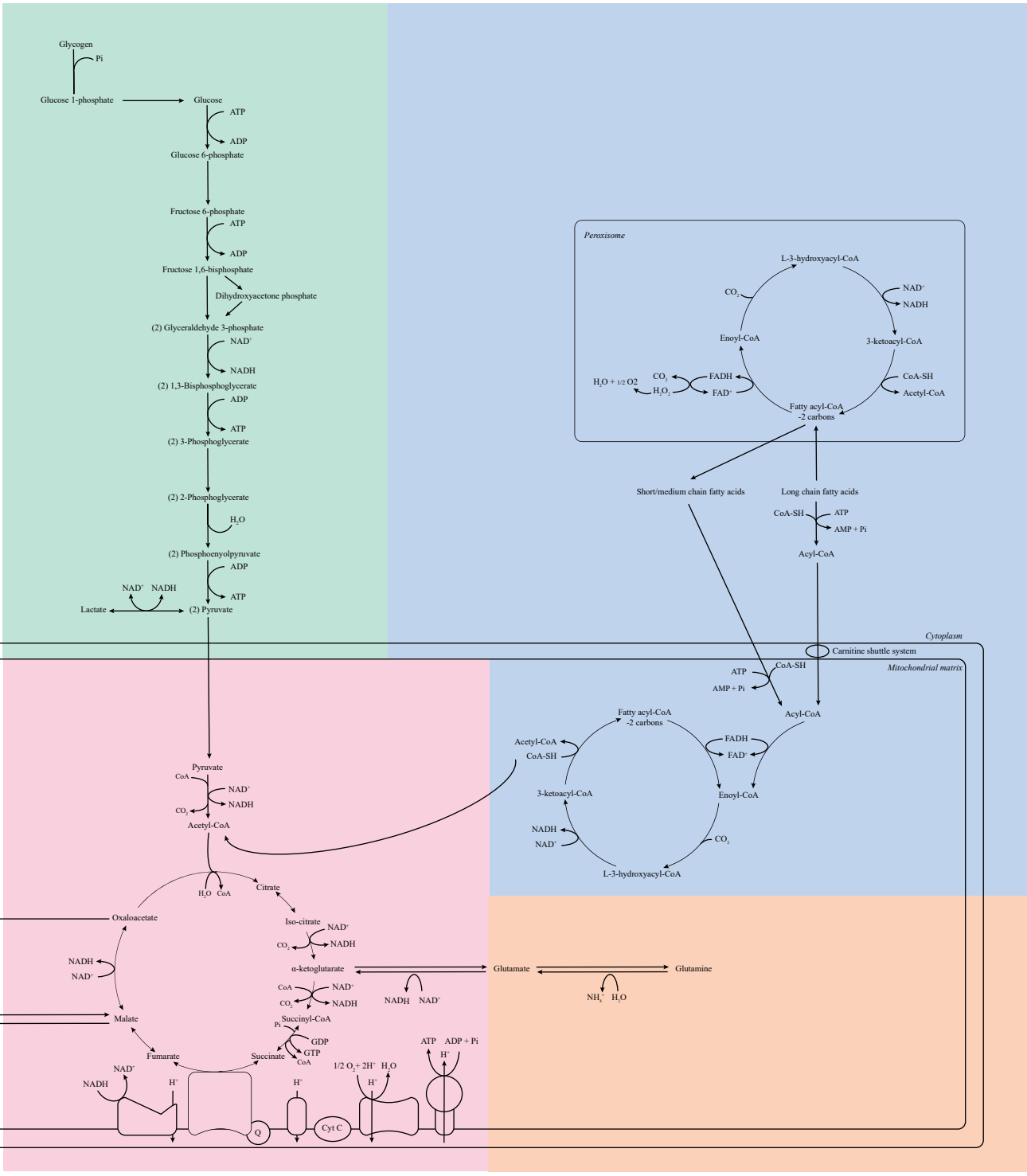
Introduction

Ischemia-reperfusion injury (I/R injury) describes the increase of tissue injury following oxygenated reperfusion with blood of a previously ischemic tissue or organ after restoration of normothermic conditions. Clinically, I/R injury is a contributor to organ damage following transient hypoxic insults, i.e. myocardial infarction and stroke, as well as for the non-immunologic graft damage after transplantation. Although numerous strategies to prevent or alleviate I/R injury have been reported, with some of them effective in animal models, unfortunately, none of these therapies have been successfully translated to the clinical setting.[1-3]

Using human kidney transplantation as an example of predicted ischemia and reperfusion, and delayed graft function as its clinical read-out, we have systematically studies the mechanisms reported to be of relevance in I/R injury.[4] In a number of studies, no primary role has been observed of oxidative damage, complement activation or inflammation [5-7], instead, strong indications have been found for the metabolic origin of I/R injury. Since similar conclusions were reached by other groups [8,9], we considered an attempt to map the metabolome of clinical I/R injury as relevant. The results of this evaluation have identified a distinct discriminative metabolic signature for I/R injury.[4] This signature points at the presence of a persistent postreperfusion metabolic paralysis as indicated by persistent (>30 minutes) post-reperfusion ATP/GTP catabolism, tricarboxylic acid (TCA) cycle defects, and a comprehensive activation of catabolic routes. Against this background, the picture emerges that whilst I/R injury may be triggered by oxidative damage[9], clinical I/R injury is actually driven by reductive stress. To be more specific, the compensatory catabolic state initiated by high-energy phosphate depletion results in a parallel production of reducing equivalents, i.e. NADH and $FADH_2$. Whilst these equivalents are normally oxidized through the electron transport chain (ETC) (Complex I and II, respectively) or by cytosolic LDH (glycolysis), the excess reductive load and apparent I/R injury -related defects in the oxidative routes may result in accumulation of reducing equivalents and thus cause reductive stress (Figure 1). In this article, we have evaluated the processes described that will contribute to reductive stress in the context of I/R injury and provide better insight in potential clinical therapeutic strategies that can be helpful in restoring the redox balance.

Figure 1. NADH NAD metabolism.




Glycogen
Pi
Glucose 1-phosphate
Glucose
ATP
ADP
Glucose 6-phosphate
Fructose 6-phosphate
ATP
ADP
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate
(2) Glyceraldehyde 3-phosphate
NAD+
NADH
(2) 1,3-Bisphosphoglycerate
ADP
ATP
(2) 3-Phosphoglycerate
(2) 2-Phosphoglycerate
H2O
(2) Phosphoenyolpyruvate
ADP
ATP
NAD+ NADH
Lactate
(2) Pyruvate
Peroxisome
L-3-hydroxyacyl-CoA
CO2
NAD+
NADH
Enoyl-CoA
3-ketoacyl-CoA
H2O + 1/2 O2
CO2
H2O2
FADH
FAD+
CoA-SH
Acetyl-CoA
Fatty acyl-CoA
-2 carbons
Short/medium chain fatty acids
Long chain fatty acids
CoA-SH
ATP
AMP + Pi
Acyl-CoA
Cytoplasm
Carnitine shuttle system
Mitochondrial matrix
ATP
CoA-SH
AMP + Pi
Acyl-CoA
Fatty acyl-CoA
-2 carbons
Acetyl-CoA
CoA-SH
FADH
FAD+
3-ketoacyl-CoA
Enoyl-CoA
NADH
NAD+
CO2
L-3-hydroxyacyl-CoA
Pyruvate
CoA
NAD+
NADH
CO2
Acetyl-CoA
H2O CoA
Citrate
Oxaloacetate
Iso-citrate
NAD+
CO2
NADH
NADH
NAD+
α-ketoglutarate
Glutamate
Glutamine
CoA
NAD+
CO2
NADH
NADH NAD+
NH4+ H2O
Malate
Succinyl-CoA
Pi
GDP
GTP
CoA
Fumarate
Succinate
ATP ADP + Pi
H+
NAD+
NADH
H+
H+
1/2 O2 + 2H+ H2O
H+
Q
Cyt C

Excessive NADH and $FADH_2$ formation in ischemia-reperfusion injury

The metabolome of renal I/R injury (clinically expressed as delayed graft function) is summarized in Table 1. This I/R injury (delayed graft function-specific) metabolome is hallmarked by a comprehensive activation of oxidation routes including glycolysis, fatty acid β-oxidation, glutaminolysis, and oxidation of branched chain amino acids. The latter presumably reflecting activation of autophagy.[4] The molecular clues for this catabolic signature are illustrated in Table 1. Oxidation of these 'fuel' sources is paralleled by a concomitant production of reducing equivalents (NADH and $FADH_2$) which attribute to an increase of the reductive load.

Disturbed NAD^+ regeneration and TCA cycle defects

Within the physiological context, redox-neutrality is maintained by oxidation of NADH and $FADH_2$ in the mitochondrial ETC (donating their electrons to complex I and II, respectively), and NADH by cytosolic LDH (oxidation of pyruvate to lactate

Table 1. Metabolic signature of clinical renal ischemia reperfusion injury (delayed graft function) based on arteriovenous differences (IN: uptake by the graft from arterial blood, OUT: release by the graft in the venous effluent) and tissue biopsies (DOWN and UP: tissue contents lower respectively higher than in control kidneys without ischemia reperfusion injury). * from glutaminolysis; # intermediates of branched chain amino acid oxidation.

Process	Flag		
Impaired ATP/GTP re-synthesis	(Hypo) Xanthine	OUT	
	Tissue phospho-creatine	DOWN	
β-oxidation	Medium chain fatty acid	IN	$NAD^+ \rightarrow NADH$
	Tissue ß-hydroxybutyric acid	UP	$FAD \rightarrow FADH_2$
Activated glycolysis	Pyruvate	OUT	$2\ NAD^+ \rightarrow 2\ NADH$
	Phosposerine	OUT	$NADH^+ \rightarrow NAD^+$
	Lactate	OUT	
	Alanine	OUT	
Glutaminolysis / Impaired malate-glutamate shuttle	Glutamate	OUT	$NAD^+ \rightarrow NADH^*$
	Tissue glutamate	DOWN	
	Aspartate	OUT	
Autophagia (branched chain amino acids)	Isovalerylcarnitine #	OUT	$NAD^+ \rightarrow NADH$
	Butyrylcarnitine #	OUT	$NAD^+ \rightarrow NADH$
TCA cycle entry defect	Acetylcarnitine	OUT	
	Tissue acetylcarnitine	UP	
TCA cycle defect	α-ketoglutarate	OUT	$NAD^+ \rightarrow NADH$
	Tissue succinate	DOWN	

(glycolysis)).[10, 11] An increased blood lactate/pyruvate ratio in grafts with delayed graft function signals an impaired redox status. While this imbalance may directly link to the comprehensive activation of catabolic pathways (increased reductive load), compelling evidence also exists for defect(s) in the mitochondrial respiratory chain.
Multiple studies have reported a defect or decreased activation of particularly complex I in the context of I/R injury.[12-15] This defect might be (partially compensated) by the recruitment of cytosolic NADH through the malate-aspartate shuttle and LDH. Yet, the impaired lactate/pyruvate ratio from renal grafts with I/R injury implies a reductive load that exceeds the capacity of LDH [4, 16], whereas post-reperfusion aspartate release points to interruption of the malate-aspartate shuttle.

System overload

As a consequence of the findings mentioned above, it appears that I/R injury may be driven by a 'system overload' caused by a wider activation of catabolic pathways, a concomitant reductive burden (Table 1), and an impairment of the oxidative machinery that results in an impaired reduction to NAD^+ and FAD; all in combination, resulting in a condition of profound reductive stress. The existence of this system overload is best illustrated by the release of acetyl-carnitine and α-ketoglutarate from kidney grafts suffering from I/R injury. Acetyl-carnitine and isovaleryl-carnitine release imply acyl production that exceeds the oxidative capacity of the TCA cycle. Specific release of α-ketoglutarate but not its TCA cycle oxidation products (as indicated by low tissue succinate levels) implies a defect at the level of α-ketoglutarate dehydrogenase, and to an increased production of α-ketoglutarate (glutaminolysis, oxidation of branched-chain amino acids). Activity of the α-ketoglutarate dehydrogenase complex relies on specific cofactors such as TTP, CoA, lipoate, and importantly FAD and NAD^+ as the final reducing equivalent (17). Critical reliance on these co-factors implies a vicious circle that sustains a situation of reductive stress.

Potential therapeutic strategies

Based on these considerations, two potential therapeutic strategies for clinical I/R injury can be considered. First, reducing the reductive burden (NADH and $FADH_2$ production), and second, boosting NAD^+ levels, either by supplementation of NAD^+ precursors or by activation of enzymes regulating NAD^+ synthesis.

The first strategy is based on the observation that the metabolome of early I/R injury is characterized by futile substrate fluxes. In fact, data show that comprehensive activation

of catabolic routes (possibly in response to the exhaustion of high energy phosphates) exceeds the oxidative capacity of the TCA cycle and LDH. Consequently, a logical strategy may be to reduce the futile carbon-fluxes. Such a strategy is supported by experimental evidence showing that removal of fatty acids, lactate and insulin from the perfusate restores the [NAD^+]/[NADH]ratio and facilitates ex-vivo recovery of mouse hearts.[18] On theoretical grounds, it could be speculated that inhibition of β-oxidation will be the most effective strategy in the context of renal I/R injury since β-oxidation associates with the highest reductive load. In this context, a targetable candidate is L-3-hydroxyacyl-CoA dehydrogenase which is inhibited by acetoacetyl-CoA (intramitochondrial inhibition) or perfluorodecanoic acid (intraperoxisomal inhibition).[19] Other, β-oxidation independent strategies include the use of glutaminase inhibitors (glutaminolysis) and glycolysis inhibitors (for the inhibition of excessive glycolysis).[20, 21]

The second and non-exclusive strategy is restoration of NAD^+ levels by administration of NAD^+ precursors, or by activation of NAD^+ biosynthetic enzymes. Although supplementation of various NAD^+ precursors (nicotinamide riboside with or without pterostilbene) has been found effective in the clinical setting (i.e. healthy individuals and patients suffering from acute kidney injury) [22-25], its translation to the I/R injury setting can be considered as challenging. In fact, clinical trial data indicate that augmentation of cellular NAD^+ levels only occur hours or even days after its oral administration. [22-25] Given that the metabolic collapse in I/R injury occurs within minutes of reperfusion, it is unlikely that these indirect (time and energy requiring 'booster' strategies will effectively improve the redox balance in the acute phase of I/R injury, although it cannot be excluded that the strategy may accelerate metabolic recovery. Moreover, NAD^+ boosting therapies might be considered as a prevention strategy for an expected I/R injury, such as in organ transplantation or in planned major surgery with arterial cross-clamping as well as cardiothoracic surgery. Similar considerations also apply to the pharmacological induction or activation of NAD^+ biosynthetic enzymes.[11, 26] Reportedly, NAD^+ boosting can be achieved by overexpression of the NAD^+-synthetic enzymes NAMPT and NMNAT, or by direct enzyme-activation with use of pharmacologic compounds such as P7C3 and SBI-797812.[11, 26-31] However, considering the prolonged response times and the reliance on agile gene translation and transcription, it is unlikely that this strategy can be used as a rescue therapy for I/R injury.

Conclusions

Whilst oxidative damage is widely considered as the key driver of I/R injury, clinical observations suggest that a post-reperfusion metabolic paralysis caused by reductive stress as the actual effector mechanism of I/R injury. The apparent inability of cells to

generate high energy phosphates results in exhaustion of the ATP/GTP pool, causing a cataplexic state that interferes with cellular homeostasis. Depletion of the high energy pool triggers a comprehensive compensatory activation of catabolic pathways (i.e. glycolysis, fatty acid β-oxidation, autophagia and glutaminolysis) in an apparent more or less futile attempt to drive ATP generation. Activation of these catabolic pathways results in a concomitant increased reduction of NAD^+ to NADH and FAD to $FADH_2$, that cannot be compensated by the mitochondrial oxidative machinery. Based on these observations we suggest to focus on strategies that aim to alleviate clinical I/R injury by inhibiting processes that excessively produce NADH and $FADH_2$ (e.g. medium chain fatty acid β-oxidation) or by promoting preventive exogenous replenishment of the NAD^+ pool to restore the redox balance.

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