Ischemia-Reperfusion Injury Driving Oxidative Stress in Organ Transplantation

Subjects: Transplantation

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During organ procurement, redox-stress triggered ischemia-reperfusion injury (IRI) is inevitable, which in addition to pre-existing damage negatively affects such organs.

Ischemia-reperfusion injury redox-stress organ transplantation machine perfusion

1. Introduction

Organ transplantation remains the ultimate treatment option for terminal organ failure. However, the number of organs in demand surpasses the number of available organs leading to a significant organ shortage [1]. Owed to the implementation of advanced preservation technologies (i.e., machine perfusion), increased-risk organs from extended criteria donors (ECD) can be considered for transplantation [2][3][4][5]. However, these organs are particularly prone to additional damage during organ retrieval, preservation and transplantation. In this regard, ischemia-reperfusion injury (IRI) caused by oxidative stress and subsequent events during early reperfusion negatively affects the short- and long-term outcome after transplantation, since several molecular downstream pathways are activated, further aggravating pre-existing damage [6][7]. It could be demonstrated that machine perfusion (MP) may mitigate oxidative stress mediated injury; however, the underlying mechanisms are not fully understood at this point [8][9][10].

In addition to whole organ perfusion experiments, which are expensive, complex and sophisticated, in vitro models such as cell lines, precision-cut tissue slices (PCTS) and organoids may be helpful, when addressing specific research questions [11]. Moreover, awareness about using animals in research has increased over the past decades. Besides economic reasons, using in vitro models can eliminate animal experiments in compliance with the 3R (replacement, reduction, refinement) principles [12].

2. IRI Is the Key Event Leading to Oxidative Stress in Organ Transplantation

Broadly, the imbalance between reactive oxygen species (ROS) generated and antioxidants present is known as oxidative stress [13]. In the setting of solid organ transplantation, one of the most common ROS-related pathologies is IRI [7][11]. IRI is inherently connected to organ transplantation. It is characterized by obstructed blood flow causing ischemia during organ retrieval and preservation, followed by a reperfusion phase when the blood flow is restored in the recipient [10][14][15][16] (**Figure 1**).

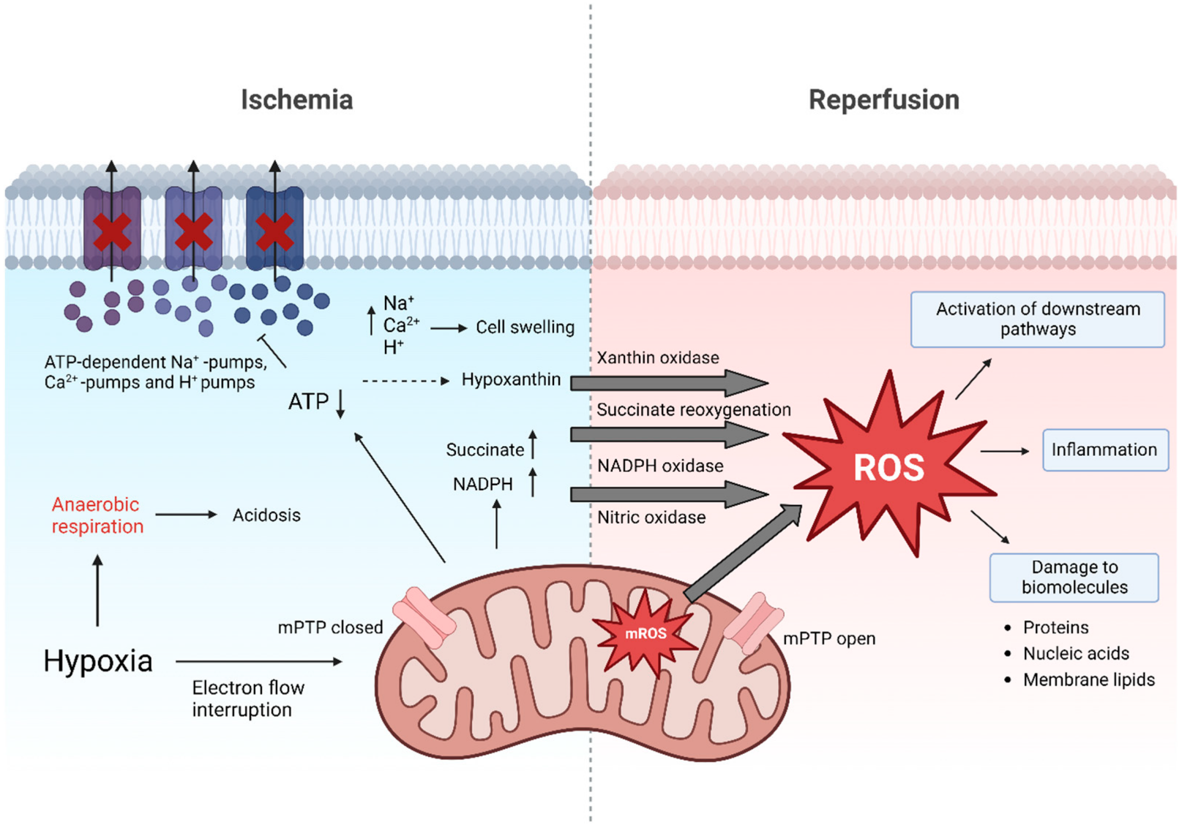

Figure 1. Molecular events of ischemia and reperfusion. During ischemia, adenosine triphosphate (ATP) levels decrease. In turn, ATP-dependent Ca2+, H+ and Na+ pumps fail, causing accumulation of ions which contributes to cell swelling. pH levels decrease leading to acidosis. Accumulation of succinate, nicotinamide adenine dinucleotide phosphate (NADPH; resulting from NADP+ and H+) and hypoxanthine during ischemia prime for excessive ROS release after reperfusion. Additionally, in mitochondria major reactive oxygen species (ROS) generation occurs. ROS cause direct damage to biomolecules but also act as signaling molecule. Besides this, opening of the mitochondrial permeability transition pore (mPTP) during reperfusion also triggers cell death by release of cytochrome c and breakdown of ATP production [5][16].

The organ retrieval process kicks off a cascade of molecular events that eventually set the basis for ROS release. The interrupted oxygen supply inhibits the mitochondrial electron transport chain, resulting in a decreased production of adenosine triphosphate (ATP) [6][17][18][19]. The subsequent shift to anaerobic metabolism leads to the retention of hydrogen (H+) ions and retained metabolic products such as lactic acid, resulting in metabolic acidosis. This decreases the cellular pH, which further leads to clumping of chromatin and impaired enzyme activity. Moreover, the ATP-dependent sodium-potassium, calcium and sodium-hydrogen pumps fail during ischemia, resulting in increased intracellular H+, sodium (Na+) and calcium (Ca2+) concentrations, which cause swelling of the cells. The partial reversal of the malate-aspartate shuttle and degradation of purine nucleotide results in an excess of fumarate, leading to a reversal of succinate dehydrogenase (SDH), ultimately causing succinate accumulation [20]. During early reperfusion it is rapidly degraded and through complex metabolic pathways it contributes to a burst in ROS production at complex I of the ETC. Additionally, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase and nitric oxidase synthase are also involved to increase ROS production under these conditions. Thus, while necessary for prolonged organ survival, the reperfusion phase in the recipient exacerbates cellular injury, which already occurred during ischemia [19][21][22][23]. Reperfusion injury is a progressive condition post-transplantation, that can last for multiple days, negatively affecting early graft function as well as long-term graft survival [19].

Free ROS can cause direct damage to biomolecules by oxidation of proteins, oxidation of nucleic acids or peroxidation of membrane lipids, ultimately resulting in cell death [6]. On the other hand, ROS are also known for their function as signaling molecules. Signaling proteins can be phosphorylated and thereby activated by ROS. Mitochondrial-activated protein kinases (MAPK), namely extracellular signal-regulated kinase (ERK1/2), c-Jun N-terminal kinase (JNK), and p38, play an important role in this cascade [24]. Phosphorylation and activation of ERK1/2 has been associated with neutrophil infiltration, necrosis, and apoptosis in rodent models of liver IRI [25][26]. The phosphorylation of JNK can lead to an increase and activation of apoptosis-promoting molecules such as Bim, Bad, Bax, and p53 [27][28][29][30]. On the other hand, phosphorylation of JNK also causes downregulation of survival signals involving STAT3 [28][30]. After reperfusion, phosphorylation and hence activation of p38 is initiated, which is directly related to apoptosis and necrosis. Its activation leads to an increase in adiponectin, which in turn enhances ROS release, resulting in tissue damage [6]. Further, apoptosis is induced by the release of cytochrome c, which in turn activates caspase-9, resulting in caspase-3 induced apoptosis. Moreover, a cascade of proinflammatory signaling pathways is induced by oxidative stress including the generation of inflammasomes [6]. Consequently, different pro-inflammatory cytokines are secreted, whereas TNFα is considered as a decisive factor for further triggering the downstream inflammatory cascade. In addition to this ‘humoral answer’ of the immune system, also the innate immune system is activated by the release of damage-associated molecular patterns (DAMPs), triggering the activation of dendritic cells (DCs), macrophages and natural killer cells (NKs) [31]. These molecular processes in combination with the lower concentration of antioxidative agents, ATP depletion and calcium dysregulation are considered the main drivers of IRI [20]. However, ROS release is not harmful by definition. This dose response phenomenon characterized by a low dose stimulation and a high dose inhibition has been observed in response to many exogenous stimuli and is referred to as hormesis in the literature [32][33]. There is growing evidence, that the lack of necessary ROS is detrimental. Together with the accumulation of reductive equivalents during ischemia, the absence of ROS is responsible for reductive stress. In this regard, three of the major couples of the cellular redox network are NAD+/NADH, NADP+/NADPH and GSH/GSSG. Like oxidative stress, also reductive stress contributes to the overall redox stress resulting in impaired cellular functions [34][35].

2.1. Molecular Mechanisms Counteracting Oxidative Stress

In order to counteract ROS, organisms exhibit their own enzymatic and non-enzymatic antioxidant defense systems. Since they may not be sufficient for averting oxidative stress, several regulatory pathways to counter it exist. They may serve as targets for treatments, which will be discussed further below [36]. One of the most important transcription factors in this regard is the Keap1-Nrf2 pathway [37]. Nuclear factor-erythroid-2 related factor

(Nrf2) is a ubiquitously expressed transcription factor regulated by the repressor protein Kelch-like ECH associated protein1 (Keap1). Upon oxidative stress, Keap1 dissociates from Nrf2, and Nrf2 can subsequently enter the nucleus and attaches to antioxidative response element (ARE). In turn the transcription of antioxidative enzymes like superoxide dismutase (SOD) or glutathione peroxidase (GPx) is induced [37]. Moreover, mammalian target of rapamycin (mTOR), which is part of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway, as well as forkhead transcription factor O (FOX) have also been described as part of the antioxidant regulatory system [38][39]. Additionally, proteins regulated by the transcription factor nuclear factor-κB (NFκB) can be activated upon degradation of inhibitor of κB (IκB) to regulate the amount of ROS in the cell [40]. However, in the context of IRI, the role of NFκB is quite controversial. For instance, activation of NFκB in the liver has been shown to reduce hepatic IRI injury and facilitate orthotopic liver transplantation [41], while another group observed protection against hepatic IRI injury upon inactivation of NFκB [42].

2.2. Biomarkers to Study Oxidative Stress

As direct contributors to oxidative stress, ROS should be considered as potential biomarkers [6][21]. Direct detection would allow for quantification of oxidative stress. However, due to the short half-life of ROS, this is currently a very complex method [43]. Instead of tracking ROS itself, their effects on biomolecules can be detected. Alterations in expression or formation induced by ROS can be used as valuable surrogate biomarkers for oxidative stress. Roughly, these molecules can be categorized as follows: endogenous antioxidants, lipid peroxidation, oxidative protein changes and nucleic acid oxidation [44]. Representative examples are listed in **Table 1** and discussed in the following sections.

Table 1. Potential biomarkers for oxidative stress.

Category	Biomarker	Examples	Analysed Material	Detection Methods	Reference
Endogenous Antioxidants	CAT	SCS vs. HMP of Human Kidneys	Perfusate	Enzymatic activity measurement	[45]
		Isolated perfused Rat Heart	Tissue	Enzymatic activity measurement	[46]
	SOD	SCS vs. HMP of Human Kidneys	Perfusate	Enzymatic activity measurement	[45]
		Patients with coronary artery by-pass grafting surgery	Serum	Enzymatic activity measurement	[47]
		Reperfusion of rat kidney	Tissue	Biodiagnostics assay kit	[48]
		Isolated perfused Rat Heart	Tissue	MTT Assay	[46]

Category	Biomarker	Examples	Analysed Material	Detection Methods	Reference
	GPx	SCS vs. HMP of Human Kidneys	Perfusate	Enzymatic activity measurement	[45]
Lipid Peroxidation	MDA	SCS vs. HMP of Human Kidneys	Perfusate	HPLC, ELISA (MDA-586 kit)	[45]
		Reperfusion of rat kidney	Tissue	Biodiagnostics assay kit	[48]
		Isolated perfused Rat Heart	Tissue	Conjugated to TBARS – Absorbance at 535nm	[46]
		Langendorff-perfused rat hearts	Tissue	HPLC/UV-Vis	[49]
	TBARS	SCS vs. HMP of Human Kidneys	Perfusate	Fluorometric Assay	[45]
		Isolated perfused Rat Heart	Tissue	TBARS-Assay	[46]
	F2 Isoprotanes	Transplanted Human Kidney	Plasma	Radioimmunoassay	[50]
		Reperfusion of Porcine Liver	Plasma	LQ/MS/MS	[51]
Protein Oxidation	Nitrotyrosine	Human Donor Livers before vs. after Transplantation	Tissue	Western Blot Analysis; Immunohistochemical Localization	[52]
		Reperfusion of Mice Kidney	Tissue	Western Blot Analysis	[53]
	Protein Carbonyl	Transplanted Human Kidneys	Plasma	DNPH Method	[54]
		NMP Porcine Kidney	Plasma	HPLC, ELISA, Immunoassays	[55]
		Langendorff-perfused rat hearts	Tissue	HPLC/UV-Vis	[49]
Nucleic Acid Oxidation	8-oxoguanine	HMP vs. SCS of canine hearts	Tissue	IHC	[56]
	8-hydroxy-2′-deoxyguanosine	Transplanted Human Kidneys	Plasma	ELISA	[54]

Category	Biomarker	Examples	Analysed Material	Detection Methods	Reference
		Patients with coronary artery by-pass grafting surgery	Serum	ELISA	[47]
		Normothermic hepatic Ischemia/Reperfusion Model of Rats	Plasma Tissue	HPLC, IHC	[57]

and SCS … xpression [58] … Moreover,

Abbreviations: CAT: catalase; DNPH: 2,4-Dinitrophenylhydrazine; ELISA: Enzyme-Linked Immunosorbent Assay; GPx: Glutathione peroxidase; HMP: Hypothermic Machine Perfusion; HPLC: High Pressure Liquid Chromatography; IHC: Immunohistochemistry; LQ: Liquid Chromatography; MDA: Malondialdehyde; MS: Mass Spectrometry; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMP: Normothermic Machine Perfusion; SCS: Static Cold Storage; SOD: Superoxide Dismutase; TBARS: thiobarbituric acid substance

Organisms possess defense systems against free radicals, one being facilitated by antioxidant enzymes [13]. These can be quantified and serve as biomarkers. Catalase (CAT) is an enzyme found in almost all living organisms that are exposed to oxygen. Within the field of transplantation, it is most widely used to assess oxidative stress [59]. Another antioxidant is SOD, an enzyme group that acts as a crucial part of the antioxidant defense against highly reactive superoxide radicals. It is responsible for splitting (dismutation) of H_2O_2 [60]. GPx can reduce H_2O_2 or organic peroxides to water and alcohol with the presence of glutathione and is subsequently converted to oxidized glutathione [44].

2.2.2. Lipid Peroxidation

It is known that ROS can promote the formation of fatty acid radicals [58]. These unstable fatty acid radicals can subsequently react with molecular oxygen to form peroxides. Moreover, lipid peroxidation products can lead to the synthesis of, for instance, malondialdehyde (MDA) [61]. MDA and the reactive thiobarbituric acid substance (TBARS) are considered basic markers of lipid peroxidation, potentially serving as biomarkers [62]. Additionally, isoprotanes serve as valuable markers, where F2 and F4 isoprotanes should be distinguished. F2 isoprotanes are formed by free radical catalyzed peroxidation of arachidonic acid, whereas F4 is a product of the same reaction of docosahexaenoic acid. It is also interesting to note that F4 isoprotanes exert a strong anti-inflammatory effect, which underlines the link between oxidative stress and inflammation [63][64].

2.2.3. Redox Modification of Proteins

When it comes to protein changes due to oxidative stress, 3-nitrotyrosin is considered as one of the most promising biomarkers [44]. Nitration of protein-bound and free tyrosine by ROS leads to the formation of this molecule. Besides nitrotyrosines, protein carbonyls are also widely used as biomarkers for oxidative stress [65][66].

2.2.4. Nucleic Acid Oxidation

Oxidative DNA damage, mostly caused by the hydroxyl radical, generates a variety of base and sugar modification products [6][67]. DNA damage caused by hydroxyl radicals occurs much less frequently than oxidative protein changes. However, the consequences of nucleic acid oxidation, such as mutations, are considerably more harmful. Although hydroxyl radicals can react with all purine and pyrimidine bases as well as with the deoxyribose backbone, the major products of oxidative nucleic acid changes are 8-oxoguanine and 8-hydroxy-2′deoxyguanosine [68].

Detection of those biomarkers can be performed in tissue samples and plasma, serum or perfusate. The selection of suitable biomarkers is depending on the study and may not rely on a single analysis method rather than on supplementary methods. Physiological levels of antioxidative enzymes like SOD and GPx and their increase in response to oxidative stress may be a more sensitive method. In contrast, evaluation of damage to biomolecules require excessive oxidative stress and may be only detected in more severe forms of oxidative stress induced damage.

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