What we need to know about lipid-associated injury in case of renal ischemia/reperfusion

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Running Title: Lipid-associated injury in renal ischemia/reperfusion

Keywords: ischemia/reperfusion, lipid metabolism, lipotoxicity

Abstract

Renal segmental metabolism is reflected by the complex distribution of the main energy pathways along the nephron, with fatty acid oxidation preferentially used in the cortex area. Ischemia/reperfusion injury (IRI) is due to the restriction of renal blood flow, rapidly leading to a metabolic switch towards anaerobic conditions. Subsequent unbalance between energy demand and oxygen/nutrient delivery compromises kidney cell functions, resulting to a complex inflammatory cascade including the production of reactive oxygen species (ROS). Renal IRI especially involves lipid accumulation. Lipid peroxidation is one of the major events of ROS-associated tissue injury. Here, we briefly review the current knowledge of renal cell lipid metabolism in normal and ischemic conditions. Next, we focus on renal lipid-associated injury, with emphasis on its mechanisms and consequences during the course of IRI. Finally, we discuss preclinical observations aiming at preventing and/or attenuating lipid-associated IRI.

Ischemia/reperfusion (I/R) injury (IRI) is the leading cause of acute kidney injury (AKI). The accumulation of lipids in renal parenchyma, also known as lipotoxicity (48), is one of the multifactorial processes occurring in IRI (63). We briefly summarize renal lipid metabolism in normal and ischemic conditions in order to help identify novel strategies to prevent lipotoxicity.

Renal lipid metabolism in normal and ischemic conditions

The metabolic pathways are heterogeneously compartmented along the nephron (19). Fatty acid oxidation (FAO) is the preferential source of kidney fuel (4, 29) and is active in all parts of the nephron (19, 4). Fatty acids (FA) are provided to renal cells from (51) (i) extracellular uptake, especially *via* FA transport proteins (such as CD36/Fatty Acid Translocase (29) or Fatty Acid Transporter Protein 1,2 or 4 (22)); (ii) *in situ* cytosolic synthesis (27); or (iii) release from intracellular processes, including triglyceride (TG)/phospholipid (PL) hydrolysis. The relative contribution is not fully established (47). Renal epithelial cells are also exposed to FA attached to albumin, with exacerbated toxicity in proteinuric kidney diseases (4, 15). FA can be converted to fatty acyl-CoA (FA-CoA) which enter the inner mitochondria matrix *via* the carnitine palmitoyl transferase (CPT) system (51). There, FA-CoA is catabolized by cyclic FAO. In addition, peroxisomes metabolize very-long-chain FA in proximal tubule (PT) cells, as suggested by PT-restricted expression of the rate-limiting peroxisomal enzyme, acyl-CoA oxydase (ACOX) (55). FA can also be stored as TG **(Figure 1)**.

IRI results from a transient interruption of renal blood flow, leading to a switch from aerobic to anaerobic metabolism (57). Ischemia rapidly induces the inhibition of FAO-associated enzymes in both mitochondria and peroxisomes (46), as well as the reduction of CPT-1 activity (25). Changes in kidney metabolomic profiles during IRI emphasize the alteration of metabolic pathways affecting glycolysis, tricaboxylic acid (TCA) cycle and lipid metabolism (28, 56). Reperfusion is characterized by a sudden increase in oxygen concentration, which results in an increased production of reactive oxygen species (ROS) – albeit in species-associated variability (57, 62). I/R-generated ROS have (i) cell-specific and (ii) concentration-dependent impact within the renal tissue, probably related to the differential redox status between tubular and interstitial cells (31). Lipid peroxidation is a destructive process, either directly by damaging membrane structure or indirectly by releasing toxic reactive products, such as lipid aldehydes (6).

Mechanisms of lipid-associated injury

Lipotoxicity refers to "accumulation of excessive lipids in non-adipose tissues, leading to cell dysfunction or death (48)". This process has been reported in several forms of AKI, especially in IRI (14, 37, 53, 63, 68). The relative contribution of lipotoxicity to the multifactorial pathogenesis of IRI remains unknown (58). Accumulation of cholesterol (63, 67) and TG (63, 68) has been documented. TG are reported as nontoxic *per se* but are reservoir of free FA (4, 20, 58). Furthermore, diacylglycerol (DG) and ceramide result from failed esterification or breakdown of TG (58). Thus, cell overload by free FA and downstream metabolites represent the main determinants of lipotoxicity (20, 58) .

Lipid accumulation in renal IRI is not fully understood **(Figures 1 and 2)**. On the one hand, a decrease in mitochondrial and peroxisomal FAO has been described during ischemia (46) and reperfusion (21) periods. On the other hand, an increase of PL hydrolysis (56), FA uptake (27, 68, 70) and lipid synthesis (27, 70) has been suggested**.** These cascades cause a mismatch between FA availability *versus* utilization. TG accumulation may not only result from alterations in TG and FA synthesis, but also from FA uptake and TG catabolism (27). PL degradation during IRI arises from various processes (57), such as enhanced cytosolic, mitochondrial and microsomal phospholipase A_2 (PLA_2) enzymatic activities (41) and [Ca^{2+}] alteration (60). However, total plasma membrane (PM) PL biomass appears well preserved *ex vivo* after PT hypoxia (69).

Lipid accumulation in kidneys is either protective or toxic depending on the time course of IRI and, therefore, the duration and extent of lipid overload (63). Initially, cholesterol and TG accumulation may be considered as protective in stabilizing PM and buffering free FA (34, 64, 66, 68). In an murine model of renal IRI, ischemia acutely results in a 3- to 4-fold increment in renal cortex [cholesterol ester], which lasted for up to 2h *post* reperfusion and which is mostly due to an increased flux of free cholesterol to the endoplasmic reticulum (67). However, profound and sustained adenosine triphosphate (ATP) depletion hampers the esterification of free FA to TG (57). TG progressively accumulate in the renal cortex after ischemia. TG levels are already significantly increased at 1 day *post* ischemia, with a peak at 1 week and a 3-week plateau (63). Such an ongoing accumulation of lipids may partially contribute to the evolution from AKI to CKD after IRI (29, 51, 63). Additionally, metabolomics have reported elevated levels of glycerol at early IRI time-points, suggesting TG lipolysis as a source of free FA (56). Hence, when cell capacity for TG storage is overpassed or in case of TG hydrolysis (48), lipid-induced cell dysfunction or death could theoretically occur through multiple pathways (48): (i) protein acylation, (ii) opening of the

mitochondrial permeability transition pore (3, 11, 43) and altered mitochondrial energy coupling (11, 43); (iii) membrane damage (37), (iv) release of proinflammatory/ proapoptotic factors and activation of apoptotic pathways; (v) ferroptosis (1, 33, 39); and (vi) increased cellular oxidative (23, 26, 49) and reticulum endoplasmic (RE) stresses (30). Particularly, the cell death process called ferroptosis is associated with lipid peroxidation and is characterized by the loss of activity of glutathione peroxidase 4 (Gpx4), a key enzyme in the repair of PM (13). The functional loss of Gpx4 results in an iron-dependent lethal accumulation of lipid hydro-peroxides (1). Lipid metabolism is closely related to cell sensitivity to ferroptosis since the accumulation of polyunsaturated FA influences the extent of lipid peroxidation and, in turn, ferroptosis (54). In addition, high levels of free FA may result in their auto-oxidation to lipid peroxides, secondary to the overwhelming of RE metabolism capacity (46). The level of lipid peroxides has been reported 250% higher in ischemic *versus* control kidneys in a rat model of IRI (46). Moreover, FA accumulation is observed in persistently impaired ATP production by mitochondria (3, 10, 12, 59). Non-esterified fatty acids (NEFA) may function as protonophoric uncouplers of oxidative phosphorylation, as well as openers of the mitochondrial permeability transition pore (10, 61). A decrease in mitochondrial membrane potential and an abrogation of mitochondrial proton gradient are both involved in NEFA-induced protonophoric mitochondrial uncoupling (9, 10, 12). Finally, PLA_2 also plays a key role in ATP recovery following renal IRI. In an *in vitro* model using PT segments exposed to hypoxia/reoxygenation, exposure to exogenous PLA_2 lowered ATP concentration and ATP/ADP ratio by 40 and 70%, respectively. This effect was most likely due to the release of arachidonic acid from cell membranes (65).

Strategies to prevent lipid-associated injury in renal ischemia/reperfusion

The general approaches for the prevention or treatment of lipotoxicity include measures that (i) decrease the global lipid content in target tissues by increasing lipid catabolism and/or lipid excretion; (ii) transfer lipids towards adipose tissue; and (iii) target critical pathways of FA-induced cell death (48) **(Figure 2)**.

Agonists of peroxisome proliferator-activated receptor α (PPARα) have been widely investigated in renal IRI. PPARα are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily, of which target genes are involved in lipid metabolism (18). In renal IRI, PPARα expression decreases (52). In preclinical studies, PPARα agonists, like fibrates, are nephroprotective (8, 32, 44, 52). They help maintain FAO with FA as major source of energy production, as well as prevent lipid accumulation and peroxidation (36). In

rats, administration of PPARα activators 5 days prior to renal I/R improved renal function, in association with an increased mRNA and protein levels of ACOX and cytochrome P4A1 (45). In a model of renal IRI in KAP2-PPARα transgenic mice, testosterone-induced up-regulation of PPARα protected kidney function and morphology, notably *via* reduced formation of lipid peroxidation products (32). Recently, polyacetylene glycoside has been reported to increase both CPT-1 and PPARα expression in hypoxic tubular epithelial cells and mouse ischemic kidneys, thereby leading to FAO enhancement and reduced lipotoxicity (70). Nevertheless, the putative nephroprotection of fibrate-induced PPARα modulation in the clinical settings of renal IRI remains unproven.

Another promising candidate concerns propinoyl-L-carnitine (7, 16, 24), a short-chain acyl derivative of L-carnitine, which may (i) help restore tissue carnitine, the essential cofactor for free FA uptake into mitochondrial matrix, and (ii) replenish mitochondrial TCA intermediates (38). In an *ex vivo* rat model of IRI, ischemic preconditioning with propionyl-L-carnitine attenuated renal damage. Moreover, this compounds prevented delayed graft function in a syngeneic rat model of kidney transplantation (38). Interestingly, in addition to attenuated oxidative stress and improved energy metabolism, pretreatment with L-carnitine significantly decreased the hydrolysate products of PL, including lysophosphatidylcholine and free FA. These observations suggest a decreased activity of PLA_2 (35). Similarly, the upregulation of CPT-1 activity by C75 compound alleviated renal IRI in rats (25).

Other pharmacological interventions aiming at reducing the generation or effects of ROS may block signaling pathways contributing to lipid-associated injury (40). These may include (i) antioxidant enzymes, such as superoxide dismutase and catalase, (ii) ROS scavengers, such as tempol (5), (iii) agents preventing ROS production, such as desferrioxamine (17), and (iv) agents inhibiting ROS-generating enzymes, such as allopurinol against xanthine oxidase (5). Despite extensive preclinical research in the field of lipid peroxidation, most of the potential benefits have not yet been translated into clinical practice.

Anti-ferroptosis compounds may also represent a pharmacological strategy for ischemic conditioning (13). In a necroptosis-insenstitive murine model, the inhibition of ferroptosis by the ferrostatin derivative 16-86 resulted in reduced levels of acyl-CoA synthetase long-chain family member 4, a key enzyme of FA metabolism. Still, no benefit was observed in IRI severity (39). Conversely, Linkermann et al. showed that the administration of ferrostatin attenuated renal IRI in mice (33). Alpha-tocopherol and desferoxamine have also been successfully tested in a murine model of IRI (2, 42).

Finally, several compounds have been studied against renal lipotoxicity, with no focus on renal IRI (48), or in ischemic conditioning, with no focus on energy/lipid modulation. Among them, pharmacological activators of AMP-activated protein kinase (AMPK) are of particular interest in ischemic conditioning (50). AMPK is an energy sensor of which downstream targets include the phosphorylative inhibition of acetyl-CoA carboxylase (ACC). ACC is one of the central enzymes involved in FA homeostasis. ACC-induced carboxylation of acetyl-CoA leads to the production of malonyl-CoA, a substrate for FA synthesis but also a potent inhibitor of CPT-1 (24). One preclinical study reports a slight effect of AMPK-associated recovery of CPT-1 activity, with no effect on malondialdehyde levels (24).

Conclusion and perspectives

A better understanding of renal metabolism may help determine if and how lipid modulation can be pharmacologically targeted to develop new approaches against IRI. Combined therapies are most probably required to achieve an efficient renal conditioning. Several compounds attenuating lipid-associated injury have been tested in preclinical models of renal IRI, with promising observations. Still, well-designed prospective randomized controlled clinical trials are needed to assess their translational relevance.

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Legends of the Figures

Figure 1. Schematic overview of lipid metabolism in renal tubular cell

The normal physiology is depicted in blue and detailed in text. Briefly, processes contributing to fatty acid (FA) pool include: (i) extracellular uptake, (ii) *in situ* cytosolic synthesis or (iii) release from intracellular processes. FA can be converted to fatty acyl-CoA (FA-CoA), which enters the inner mitochondria matrix *via* the carnitine palmitoyl transferase (CPT) system. There, FA-CoA is catabolized by cyclic FA oxidation (FAO). Peroxisomes metabolize very long-chain FA. FA can also be stored into TG. AMPK is an energy sensor, with downstream targets including the acetyl-CoA carboxylase (ACC). PPARα are ligand-activated transcription factors of various genes involved in lipid metabolism.

(↓) or **(↑)** represent metabolism modulations caused by renal ischemia/reperfusion (I/R). Decreased delivery of oxygen results in the lowering of the intracellular pH (due to accumulation of lactic acid) and the [ATP] levels. I/R causes (i) a decrease of FAO with a decline in CPT- activity 1 and the inhibition of FAO-associated enzymes in both mitochondria and peroxisomes; and (ii) a downregulation of PPARα. Increased PLA2 activity and FA uptake have been reported. Under circumstances of a mild ATP reduction **(*)**, an increased synthesis of lipids may also contribute to lipid accumulation (27). Of note, the I/R-associated metabolic changes sequentially occur during I/R.

ACC; acetyl-CoA carboxylase, ACOX, acyl coA oxidase; AMPK, AMP-activated protein kinase ; ATP, adenosine triphosphate; CAT, carnitine translocase ; CPT; carnitine palmitoyl transferase; DGAT, diglyceride acyltransferase; VLCFA, very long chain fatty acid; PLA_2, phospholipase A_2; PPAR α, peroxisome proliferator-activated receptor α. Broken lines indicate multi-step processes.

Figure 2. Lipid-associated injury in renal ischemia/reperfusion, and potential pharmacological approaches

Renal ischemia/reperfusion (I/R) causes a mismatch between FA availability *versus* utilization, leading to lipid accumulation. Lipid-induced cell dysfunction/death involve multiple pathways (48): (i) protein acylation, (ii) opening of the mitochondrial permeability transition pore (3, 11, 43) and altered mitochondrial energy coupling (11, 43); (iii) membrane damage (37), (iv) release of proinflammatory/ proapoptotic factors and activation of apoptotic pathways; (v) ferroptosis (1, 33, 39) and (vi) increased cellular oxidative (23, 26, 49) and reticulum endoplasmic (RE) stresses (30). The panels represent strategies evaluated in

preclinical models to limit lipid-associated I/R injury.

AMPK, AMP-activated protein kinase ; CPT; carnitine palmitoyl transferase; FA, fatty acid; PPAR α, peroxisome proliferator-activated receptor α.