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Oxidative and Nitrosative Stress in the Ischemic Acute Renal Failure

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1. Introduction

Ischemic injury to the kidney is the most common cause of acute kidney injury. Despite intensive basic research and in critical care for decades it is still associated with high mortality rates of ~50% in the intensive care unit. It is observed in a variety of clinical situations such as cardiac arrest with recovery, organ transplantation, or heminephrectomy. Postischemic acute kidney injury is characterized by an abrupt decrease in glomerular filtration rate (GFR) (the hallmark feature of acute kidney injury), and increased renal vascular resistance that determines a persistent reduction in renal blood flow (RBF) and tubular injury. However, the pathophysiological mechanisms responsible for the postischemic renal injury and the profoundly depressed renal function remain incompletely understood. The accumulated data in the literature are compatible with the hypothesis that ischemic acute kidney injury is essentially a phenomenon of altered renal hemodynamics linked critically to endothelial cell dysfunction caused by the production of high levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to decreased nitric oxide availability as a consequence of its destruction to form peroxynitrite, associated with an intracellular energy store depletion. The oxidative and nitrosative stress will produce lipid peroxidation, oxidative DNA damage and modification and inactivation of proteins that originates an inflammatory reaction characterized by endothelial activation and injury, enhanced endothelial cell-leukocyte adhesion, leukocyte entrapment, and a reduction in microvascular blood flow mainly affecting the renal outer medulla as indicated by the marked vascular congestion typically observed in this zone of the kidney. On the other hand, and depending on the severity of renal ischemia, tubular epithelial cells will undergo a varying degree of necrosis or apoptosis with tubular obstruction followed by both an anatomical and functional recovery. The way in which vascular and tubular epithelium recover determines the final status of the renal function, ranging from full recovery to chronic renal failure and ultimately to end-stage renal disease. Because of the importance of endothelial cells in this process, emphasis will be placed on the involvement of oxidative and nitrosative stress in causing endothelial dysfunction, the sources of oxygen and nitrogen reactive species, and the interactions between them, specially superoxide anion and nitric oxide because together they form peroxynitrite, a potent oxidant and nitrosant agent. Among other factors, the severity of acute kidney injury is mainly determined by the duration of the ischemia. Special attention will be paid to the vascular and hemodynamic

changes produced in the outer medulla during renal ischemia/reperfusion, because this renal zone is physiologically nearly hypoxic. The role of heme oxygenase system and the gender differences in the susceptibility to ischemic acute renal failure will be also be revised

2. Morphologic and hemodynamic changes in ischemic acute kidney injury

In apparent disagreement with the severe impairment of renal function, histologic changes in acute kidney injury are relatively subtle, and necrosis (if present) is restricted to the outer medullary region of the kidney. Morphologic changes include effacement and loss of proximal tubule brush border, patchy loss of tubule cells with apoptosis limited to both proximal and distal tubules, focal areas of tubular dilation with distal tubular casts (consisting of Tamm-Horsfall protein and cellular debris) and areas of regeneration. Peritubular capillaries present endothelial injury with enhanced expression of adhesion molecules (e.g. intercellular adhesion molecule-1, E-selectin, P-selectin) and cell swelling that promote adhesion of platelets to endothelium, with subsequent leukocyte adhesion and adhesion of platelets to neutrophils which are then aggregated and trapped in narrow peritubular capillaries causing vascular congestion, with cessation and even reversal of blood flow (Brodsky et al, 2002; Yamamoto et al, 2002). Endothelial injury also iniciates an inflammatory response that can be enhanced by tubular cells through the generation of proinflammatory cytokines and chemotactic cytokines (Bonventre & Zuk, 2004; Friedewald & Rabb, 2004; Schrier et al, 2004; Devarajan, 2006).

Functionally, the ischemic insult is followed by an intense and persistent renal vasoconstriction that significantly reduces renal blood flow to ~50% of normal (Cristol et al, 1993; Lieberthal et al, 1989), and has dramatic consequences in the renal outer medulla due to the fact that it is physiologically on the verge of hypoxia. Through a poorly understood mechanism, this acute reduction in outer medullary blood flow is followed later by a situation of chronic hypoxia (Basile et al, 2001; López Conesa et al, 2001). The increased basal vascular tone is also accompanied by increased reactivity to vasoconstrictors and a decreased response of arterioles to vasodilators, with loss of autoregulation of renal blood flow and abnormal vascular reactivity characteristic of postischemic acute kidney injury (Bonventre & Weinberg, 2003). These changes have been attributed to altered prostaglandins synthesis, to the generation of reactive oxygen and nitrogen species, and/or to activation of inflammatory responses to ischemia and it seems to be critically linked to endothelial dysfunction and to the increased generation of reactive oxygen and nitrogen species (oxidative and nitrosative stress) with a decrease in nitric oxide availability.

3. Temporal course of ischemic acute renal failure

Clinically, ischemic acute renal failure has classically been divided into the "Initiation", "Maintenance" and "Recovery" phases (Sutton et al, 2002; Devarajan et al, 2006). Sutton et al (2002) proposed a fourth phase, the "Extension" phase.

The "Initiation" phase begins when cellular ATP content becomes depleted as a consequence of anoxia, with the resultant tubular epithelial, smooth muscle and endothelial cell injuries characterized by disruption of actin cytoskeleton that produces structural and functional tubular alterations, and renal vasculature abnormalities. The severity and extent of these injuries will be determined by the degree and duration of ischemia. From a

functional point of view epithelial and endothelial cells become "activated" up-regulating a number of cytokines and chemokines such as interleukins -1, -6, and -8, monocyte chemoatractant protein-1, and tumor necrosis factor alpha, thus triggering the inflammatory cascade. A key event in the "activation" of endothelial cells is a decrease in nitric oxide production.

The "Extension" phase is determined by two major events, a state of continued hypoxia with decreased blood flow, stasis and red and white blood cells accumulation mainly affecting outer medulla, and an inflammatory response. Thus, endothelial dysfunction in this phase plays a key role in the continued ischemia of tubular cells as well as in the inflammatory response observed in ischemic acute renal failure. As a consequence of these changes, apoptosis and necrosis of tubular cells (mainly affecting outer medulla) is observed and glomerular filtration rate continues falling.

During the "Maintenance" phase cells undergo repair (with apoptosis, proliferation and migration of cells) to re-establish and maintain cell and structure integrity with a slow improving in cellular and tubular function. Glomerular filtration rate is maintained to a level determined by the severity and duration of ischemia. Renal blood flow recovers approaching preischemic levels. During the "Recovery" phase a slowly and progressive improvement towards normality is taking place.

During all these phases the initial endothelium dysfunction and its posterior recuperation are of key importance to overall recovery.

4. Importance of renal medulla in the renal response to ischemia

4.1 Susceptibility of renal medulla to hypoxia

Many studies indicate that the severity of post-ischemic renal injury depends on the state of persistent hypo-perfusion of the renal outer medulla. The susceptibility of renal medulla to hypoxia lies in the fact that: a) renal arteries and veins run strictly parallel and in close contact with each other over long distances, allowing oxygen to diffuse from the arterial to the venous system before it has entered the capillary bed; b) tubular segments of the outer medulla have a limited capacity for anaerobic energy generation and, thus, depend on its oxygen supply to maintain active transtubular sodium and the reabsorption and secretion of solutes. These facts are particularly relevant in the tubular segments of the outer medulla (S3 segment of proximal tubules and medullary thick ascending loop of Henle) where the combination of limited oxygen supply (pO_2 < 25 mmHg) and a high oxygen demand makes the outer medulla to be physiologically on the verge of hypoxia (Brezis & Rosen, 1995; Zhang & Edwards, 2002). A variety of physiologic mechanisms are involved in protecting the outer medulla against hypoxic injury, including nitric oxide, prostaglandins, heme oxygenase-1 and adenosine, all of which enhance medullary blood flow while down-regulate active tubular transport of sodium and solutes (Brezis et al, 1989; Brezis et al, 1991; Knight & Johns, 2005; Rosenberger et al, 2006). A number of studies have shown that nitric oxide is a main regulator of medullary blood flow. Inhibition of nitric oxide production is followed by a decrease in medullary pO_2 in control animals and medullary blood flow (Cowley et al, 2003; Fenoy et al, 1995; López-Conesa et al, 2001; Nakanishi et al, 1995; O´Connor et al, 2006; Rodríguez et al, 2010; Rosenberger et al, 2006). Therefore, the

functional status of the renal medullary nitric oxide system after the ischemia-reperfusion injury is believed to be a major determinant in the development of renal failure.

4.2 Outer medulla and ischemia-reperfusion

The importance of outer medulla in the renal response to an ischemic event has been demonstrated by several studies. Basile et al (2001) observed that renal ischemia results in permanent damage to peritubular capillaries and influences long-term function. They measured a 30-50% reduction in peritubular capillary density in the outer medulla at 4, 8 and 40 weeks after ischemia and tubulointerstitial fibrosis with increased transforming growth factor-1 expression at 40 weeks. Moreover, they also demonstrated an increase in 2-pimonidazole staining (a hypoxia-sensitive marker) in outer medulla accompanied by proteinuria, interstitial fibrosis and renal functional loss. They also observed that chronic L-arginine administration in drinking water increased total renal blood flow, decreased 2-pimonidazole staining and attenuated or delayed the progression of chronic renal insufficiency after recovery from acute ischemic injury (Basile et al, 2003). On the other hand, López-Conesa et al (2001) reported that an antioxidant ameliorated the renal failure and prevented the outer medullary vasoconstriction observed after 45 min of renal ischemia, effects that seem to be dependent on the presence of nitric oxide and the scavenging of peroxynitrite. Taken together, data from these studies strongly suggest that the renal failure that follows an ischemic event is directly related to alterations in outer medullary blood flow and that these changes seems to be dependent on free radical production and nitric oxide bioavailability.

5. Free radicals in acute renal injury

Free radicals are small, diffusible molecules that have an unpaired electron and tend to be reactive and can participate in chain reactions in which a single free radical event can be propagated to damage multiple molecules. The generation of oxygen free radicals is mainly restricted to mitochondria. In a controlled process 4 electrons from the electron transport chain are added to molecular oxygen yielding two water molecules. These electrons additions generate sequentially superoxide anion, hydrogen peroxide and the hydroxyl radical before the addition of the final electron to produce water. Reactive oxygen species can be also endogenously generated from other enzymes such as NAD(P)H-oxidases, xanthine oxidases, cyclooxigenases, lipooxygenases, myeloperoxidases, or uncoupled nitric oxide synthases. Each of these free radicals is able of oxidizing surrounding biomolecules thus generating other potent oxidants such as hypochlorous acid (harnessed by phagocytes for bacterial killing) or peroxynitrite anion (formed by the reaction of equimolecular amounts of nitric oxide and superoxide). However, the idea that the effects of reactive oxygen species on cellular functions are always deleterious is no longer valid because a number of studies have demonstrated that under physiological conditions low concentrations of reactive oxygen species play an important role in the normal regulation of cell and organ function. In this regard Ignarro et al (1988) demonstrated that superoxide dismutase enhanced arterial relaxation induced by the infusion of acetylcholine, indicating that there is a physiological production of a small amount of superoxide that is normally counteracting the vasodilatory effect of nitric oxide. In the kidney, Zou & Cowley (2001) demonstrated a basal generation of superoxide anion in all renal zones with the highest

production in the outer medulla. Nitric oxide is a known renal vasodilator and acts as a natriuretic agent; superoxide has been shown to decrease renal blood flow and sodium excretion (Majid & Nishiyama, 2002; Majid et al, 2004, 2005; López et al, 2003; Makino et al, 2002; Zou & Cowley, 2001). Thus, there is evidence suggesting that superoxide is an important physiologic modulator of endogenous NO activity, counteracting the effects of nitric oxide in the kidney, and that superoxide exerts a tonic regulatory action on renal medullary blood flow.

Under steady-state conditions free radicals are effectively eliminated by antioxidant defense mechanisms that include free radical scavenging enzymes (superoxide dismutase, catalase, or glutathione peroxidase) and abundant radical scavenging chemicals (reduced glutathione, cysteine, vitamins C and E) that prevent almost completely radical chain reactions. However, when present in excess, a condition known as oxidative stress, they exert deleterious effects including lipid peroxidation, oxidative DNA damage and protein oxidation and nitration that collectively lead to progressive endothelial and tubular cells damage described in the precedent section. Generation of high levels of reactive oxygen species during renal ischemia/reperfusion have been confirmed directly (Zweier et al, 1994; Salom et al, 2007) and indirectly by measuring the effects of oxidants on lipids, proteins and DNA and by determining the beneficial effects of free radicals scavenging with antioxidant enzymes like superoxide dismutase or catalase or with antioxidants allopurinol (a xanthine oxidase inhibitor), tempol (an superoxide dismutase mimetic), N-acetyl-L-cysteine (an antioxidant), or dimethylthiourea (a hydroxyl radical scavenger)(Chatterjee et al, 2000; López-Conesa et al, 2001; Nitescu et al, 2006; Noiri et al, 2001; Tsuji et al, 2009). However, these compounds also scavenge or inhibit the formation of peroxynitrite ($ONOO^-$) a highly reactive chemical specie derived from nitric oxide and superoxide. Peroxynitrite and other reactive nitrogen species act together with other reactive oxygen species to damage cells, causing what is known as nitrosative stress.

6. Renal vascular endothelium, nitric oxide and acute renal failure

6.1 Vascular endothelium and nitric oxide in renal function regulation

The endothelium is the thin layer of cells that lines the interior surface of blood vessels, forming an interface between circulating blood in the lumen and the rest of the vessel wall. The vascular endothelium regulates vascular permeability, and modulates vasomotor, inflammatory, and haemostatic responses and nitric oxide appears to play a key role in these regulatory functions (Bird, 2011; Michel, & Vanhoutte, 2010). Nitric oxide regulates vascular tone preventing abnormal constriction, inhibits platelet aggregation, the expression of adhesion molecules at the surface of endothelial cells thus inhibiting the adhesion and penetration of white blood cells, and the release and action of endothelin-1 (Michel & Vanhoutte, 2010).

6.1.1 Nitric Oxide System

Nitric oxide is a diatomic free-radical gas synthesized from L-arginine by a family of enzymes called nitric oxide synthases. There are three mammalian nitric oxide synthases isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). They share 50–60% homology at the amino acid level and have an N-terminal oxygenase domain with heme-, L-

arginine-, tetrahydrobiopterin (BH4)-binding domains, a central calmodulin (CaM)-binding region, and a C-terminal reductase domain with NADPH, FAD, and FMN binding sites (Stuehr, 1997). Under physiological conditions, the dominant nitric oxide synthase isoform in the vasculature is endothelial nitric oxide synthase, which is dynamically regulated at the transcriptional, posttranscriptional, and posttranslational levels (see Rafikov R et al, 2011 for a comprehensible review of the posttranslational control of endothelial nitric oxide synthase). Nitric oxide synthesis requires binding of the Ca^{2+}/calmodulin complex, but also requires dimerization of endothelial nitric oxide synthase and cofactors binding for activity. In the inactive state, endothelial nitric oxide synthase is located in plasma membrane caveolae bound to inhibitory protein caveolin-1. When activated the increase in Ca^{2+}/CaM releases and dimerizes endothelial nitric oxide synthase and interacts with its associated proteins heat shock protein 90 and Akt, and cofactors in an active complex. This activation process requires phosphorylation/dephosphorylation of the enzyme at different sites of tyrosine (Tyr-81 and Tyr-657), serine (Ser-114, Ser-615, Ser-633, and Ser-1177), and threonine (Thr495). The enzymes cycles between the inactive state bound to caveolin-1 in caveolae to cytoplasma in the activated state. The production of nitric oxide in endothelium cells is induced by mechanical action (shear stress) and by agonists such as acetylcholine, bradykinin, or histamine. Nitric oxide freely diffuses through plasma membrane to the underlying smooth muscles and triggers their relaxation by stimulating soluble guanylate cyclase that increases cyclic guanosine monophosphate levels. Nitric oxide also diffuses to the endothelium surface where inhibits adhesion and aggregation of platelets, modulates the permeability of endothelium, and inhibits endothelium-leukocytes interaction by reducing the expression of adhesion molecules.

Nitric oxide also plays an important role in the regulation of the renal hemodynamic and excretory functions (Romero et al, 1992). Inhibition of nitric oxide synthesis has shown to worsen both cortical and medullary blood flow and oxygenation (Cowley et al, 2003; Brezis et al, 1991)., indicating that nitric oxide is important for the maintenance of renal blood flow after ischemia-reperfusion injury of the renal vascular bed.

6.2 Endothelial dysfunction and acute renal failure – Role of nitric oxide

Ischemia/reperfusion of the kidney is followed by endothelium dysfunction and injury that contribute to the impairment of renal perfusion and chronic hypoxia, with the subsequent epithelial cell injury and decrease in the glomerular filtration rate that are the hallmarks of acute renal failure. Endothelial dysfunction, defined as impaired vasorelaxation in response to endothelium-dependent vasodilators, has been observed during renal ischemia-reperfusion (Brezis & Rosen, 1995; Cristol et al, 1993; Erdely et al, 2003; Kher et al, 2005; Lieberthal et al, 1989; Salom et al, 1998). Cristol et al (1993) and Salom et al (1998) reported renal vasoconstriction and impairment of the vasodilator effect of acetylcholine after acute renal ischemia. They also found that the recovery of renal blood flow observed on reperfusion was prevented by the previous nitric oxide synthesis inhibition. Renal ischemia/reperfusion is accompanied by a persistent reduction in renal blood flow of greater magnitude in the outer medulla. Mechanisms involved in this persistent reduction in renal perfusion are incompletely understood, but it has been observed endothelial cell swelling and detachment with trapping of red blood cells and leukocytes that produce vascular congestion of renal microcirculation especially in outer medulla (Olof et al, 1991;

Hellberg et al, 1990a, 1990b; Mason et al, 1984; Solez et al, 1974). Endothelial dysfunction in the outer medulla could contribute to the tubular epithelial cell injury thus determining a progressive fall in glomerular filtration rate in the initial and extending phases of acute renal failure. The key role of endothelial dysfunction in acute renal ischemia was demonstrated by studies of Brodsky et al, (2002) who transplanted endothelial cells or surrogate cells expressing endothelial nitric oxide synthase into rats subjected to renal artery clamping. Implantation of endothelial cells or their surrogates in the renal microvasculature resulted in a dramatic functional protection of ischemic kidneys. These observations strongly suggest that endothelial cell dysfunction is the primary cause of the no-reflow phenomenon, which, when ameliorated, results in prevention of renal injury seen in acute renal failure.

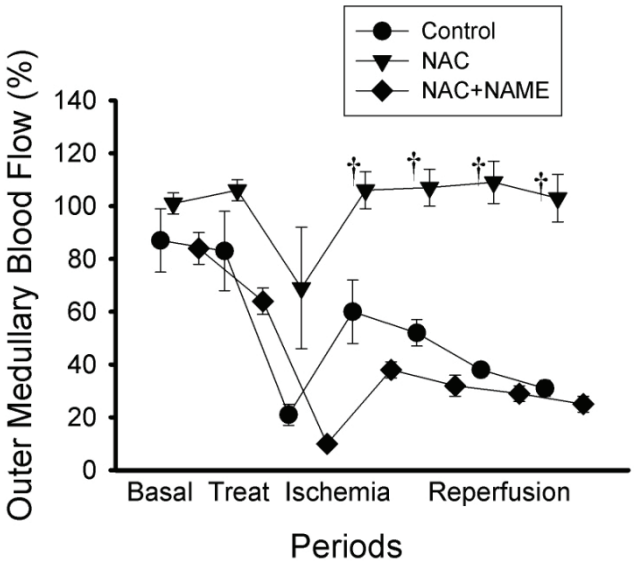

Fig. 1. Outer medullary blood flow during renal ischemia/reperfusion in SD rats. % Change from the basal period in outer medullary blood flow during renal ischemia/reperfusion (45 min occlusion of renal artery) in Sprague-Dawley rats infused (Treat.) with saline (Control), N-acetyl-L-cysteine (150 mg/kg, as a bolus, plus 715 µg/kg/min) or L-Name (10 µg/kg/min), or L-Name + N-acetyl-L-cysteine).† Significant difference from the same experimental period of the control group (López-Conesa et al, 2001)

Endothelial dysfunction is an early event that is produced when oxygen free radicals are produced on reflow. Tsao et al (1990), Tsao & Lefer (1990) and Lefer & Ma (1991) in cardiac and splanchnic ischemia-reperfusion experiments demonstrated that endothelial dysfunction was related to reoxygenation and not to reflow and that this noxious effect can be prevented when free radical scavengers are infused before reperfusion. In the kidney, an increased free radical production has been demonstrated during reperfusion in *in vitro* (Kadkhodaee et al, 1995; Paller & Neumann, 1991) and *in vivo* experiments (Haraldsson et al, 1992; Nilsson et al, 1993). There is indirect evidence showing that endothelial dysfunction

appears to be due to the generation of oxygen free radicals during reperfusion (Lieberthal, 1997; Salom et al, 1998). When infused before reperfusion, oxygen free radical scavengers exert a beneficial effect by preventing oxygen free radical production during reoxygenation (Baker et al, 1985; Hansson et al, 1990; Nilsson et al, 1993; Salom et al, 1998). In addition, the beneficial effect of some scavengers has been attributed to nitric oxide potentiation (Caramelo et al, 1996; López-Neblina et al, 1996; Salom et al, 1998), suggesting that the inactivation of nitric oxide by free radical is an important factor contributing to postischemic acute renal failure. This hypothesis was tested by López-Conesa et al, (2001) who found that N-acetyl-L-cysteine, a free radical scavenger, ameliorated the renal failure, and prevented the outer medullary vasoconstriction and the increase in plasma concentration of rhodamine 123 (index of peroxynitrite production) induced by renal ischemia. These results suggest that beneficial effects of N-acetyl-L-cysteine seem to be dependent on the presence of nitric oxide and the scavenging of peroxynitrite.

6.3 Ischemic preconditioning

Ischemic preconditioning is a phenomenon induced by brief ischemia and reperfusion periods that renders an organ more tolerant to subsequent sustained ischemia/reperfusion. In preconditioned kidneys, the sustained ischemia produces only small increases in plasma creatinine and in fractional sodium excretion, accompanied by markedly attenuated outer medullary congestion and leukocyte infiltration (Park et al, 2001, 2002). The mechanisms underlying this protective effect against injury are not well known. However, several candidates that could potentially serve as mediators of the preconditioning phenomenon have been identified. One of them, the nitric oxide synthase pathway, seems to be of importance. Park et al (2003) observed that prior ischemia results in prolonged increase in endothelial and inducible nitric oxide synthases (eNOS and iNOS). Torras et al (2002) observed that the protection afforded by ischemic preconditioning is abrogated by the inhibition of iNOS, and reproduced by a nitric oxide donor. Park et al (2003) showed that gene deletion of inducible (but not endothelial) nitric oxide synthase increases the kidney susceptibility to ischemia. In apparent contradiction, Yamasowa et al (2005) found that preconditioning in $eNOS^{+/+}$ mice markedly attenuated the renal dysfunction and improved the histological renal damage that follow ischemia/reperfusion (medullary congestion, intratubular casts or tubular necrosis). Preconditioning also prevented the marked decrease in endothelial nitric oxide synthase activity observed 6 hours after ischemia. (Yamasowa et al, 2005). The effects of preconditioning were abolished by a non-selective inhibitor of nitric oxide synthases, whereas aminoguanidine (a selective inhibitor of the inducible isoform) had no effect. A role for nitric oxide has also been reported by Jefayri et al (2000). Differences in the length of time between the first (preconditioning) and the second ischemic episode may explain differences observed between these studies. Park et al (2003) performed the second ischemia 1, 3, 4, 6, 10, or 12 weeks after the first (preconditioning) ischemia and at that time the expression of inducible but not endothelial nitric oxide synthase was significantly increased and nitric oxide levels before the second ischemia were high due to inducible isoform of the enzyme. In the study of Yamasowa et al (2005) the second ischemia was performed immediately after the preconditioning was finished (5 min). Yamasowa et al found that preconditioning prevents the decrease in endothelial nitric oxide synthase activity and prevented the increase in inducible nitric oxide synthase activity

observed after 6 h of reperfusion. As Nakajima et al (2006) observed an increased production of superoxide in the first day after the ischemia, the inhibition of inducible nitric oxide synthase could theoretically prevent the formation of high levels of peroxynitrite. This could explain why inhibition of inducible nitric oxide synthase before the ischemia reduces renal ischemia/reperfusion injury (Chatterjee et al, 2002; Walker et al, 2000) and why inhibition of inducible nitric oxide synthase before the ischemia prevents renal microvascular hypoxia and inhibition of endothelial isoform aggravates renal function (Legrand et al, 2009). The data of these studies indicate that preconditioning protects the kidney partly by increasing nitric oxide levels due to the increase in endothelial (early protecting effect) or in the inducible nitric oxide synthase activity (long lasting protective effect). The studies of Nakajima et al (2006) who observed a significant attenuation of nitrotyrosine formation, neutrophil infiltration into renal tissues, and renal superoxide production, that were significantly attenuated by the preischemic treatment with a nitric oxide donor. Thus, a better understanding of ischemic preconditioning may help to unravel the underlying mechanisms of protection that mediate this tolerance against injury.

7. Oxidative and nitrosative stress and endothelial dysfunction in ischemic acute kidney injury

Endothelial dysfunction and oxidative stress are the main pathophysiological mechanisms of several diseases such as hypertension, atherosclerosis, dyslipidemia, diabetes mellitus, cardiovascular disease, renal failure and ischemia-reperfusion injury. Reactive oxygen species can modulate cellular function, receptor signals and immune responses in physiological conditions, but when present in excess, they mediate progressive endothelial damage through growth and migration of vascular smooth muscle and inflammatory cells, alteration of extracellular matrix, apoptosis of endothelial cells, activation of transcription factors (NFkB, AP-1), and over-expression of inflammatory cytokines and adhesion molecules (ICAM-1, VCAM-1 , E-selectin). Recent evidences suggest that the major source of reactive oxygen species is the NADPH-oxidase, especially activated by angiotensin II, shear stress and hyperglycemia. The unbalance between production of free radicals and the ability to neutralize them by antioxidant systems causes a condition of "oxidative stress". Reactive oxygen species alter vascular tone by increasing concentration of cytosolic calcium and especially causing a decreased availability of nitric oxide, the principal agent of endothelial function with vasodilating action (Urso & Caimi, 2011).

Ischemia/reperfusion is accompanied by an increase in radical oxygen species, a situation known as oxidative stress. The superoxide anion formed reacts with nitric oxide and inactivates nitric oxide (endothelial dysfunction) producing peroxynitrite, a highly reactive oxidant specie that exerts profound deleterious effects on renal function. The amount of peroxynitrite and, thus, the severity of postischemic acute kidney injury will depend on the relative concentration of both nitric oxide and superoxide (Miles et al, 1996) in such a way that the higher the nitric oxide concentration, the lower peroxynitrite will be formed and less renal damage will take place after ischemia. Renal nitric oxide levels increase dramatically during ischemia decreasing to near preischemic levels on reperfusion (Figure 1). The increase in nitric oxide concentration seems to be independent of nitric oxide synthases and appears to originate in tissue nitric oxide stores that release nitric oxide

during ischemia (Salom et al, 2005). Combined with superoxide produced during ischemia, the nitric oxide increase appears to be responsible for an important part of the ensuing renal damage.

It has been reported that arterial ischemia produces an abrupt and significant increase in tissue nitric oxide concentration, which can last as long as ischemia is maintained and returns to preischemic levels during reperfusion. This phenomenon has been observed in kidney (Saito & Miyawaga, 2000) as well as in other organs (Lhuillier et al, 2003; Zweier et al, 1999). Although its physiological relevance is unclear, it may generate the high levels of peroxynitrite anion formed during reperfusion when a burst of superoxide anion reacts with the high levels of nitric oxide accumulated during ischemia (Miles et al, 1996), thus contributing to reperfusion damage. The mechanism responsible for these increased nitric oxide levels during renal ischemia is unknown, although it seems to be partially insensitive to nitric oxide synthesis inhibition, at least in liver and kidney (Lhuillier et al, 2003; Saito & Miyawaga, 2000). This is not surprising because nitric oxide synthase requires molecular oxygen. Therefore, during ischemia, nitric oxide must be released from other sources, such as tissue nitric oxide stores (Muller et al, 1996; Rodriguez et al, 2003; Sogo et al, 2000).

In the presence of oxygen, nitric oxide is synthesized from L-arginine through the action of nitric oxide synthase, and this gaseous hormone acts in the kidney by stimulating guanylyl cyclase and by inhibiting cytochrome *P*-450 (López et al, 2003). However, as soon as it is synthesized, nitric oxide avidly reacts with molecular oxygen, superoxide anion, and heme groups. The wide availability of these nitric oxide scavengers in all tissues argues against the simple diffusion-limited transport of free nitric oxide from synthase to cyclase or cytochrome. This implies that nitric oxide must be stabilized in vivo by reacting with carrier molecules that prolong its half-life and preserve its biological activity. This role may be subserved by biological molecules containing sulfhydryl groups that readily react with nitric oxide to form *S*-nitrosothiols (Stamler et al, 1992b), which are significantly more stable than nitric oxide itself and have been shown to be long-lasting and potent vasodilators. These compounds have been postulated to be biologically active intermediates in the mechanism of action of nitric oxide (Stamler 1992a). From this point of view, it has been shown that, at physiological concentrations, nitric oxide reacts with thiols in the presence of oxygen to form *S*-nitrosoglutathione (Kharitonov et al, 1995) and that nitric oxide circulates in mammalian plasma as nitrosothiols, mainly *S*-nitroso-serum albumin (Stamler et al, 1992a). The abundance of *S*-nitrosothiols in plasma compared with that of nitric oxide (3- to 4-fold) (Stamler et al, 1992a) suggests that plasma *S*-nitrosothiols may serve as a reservoir of nitric oxide, acting as an effective buffer (Lhuillier et al, 2003). This has also been shown in vascular tissue (Muller et al, 1996), where *S*-nitrosothiols are known to cause a prolonged nitric oxide-dependent relaxation (Sogo et al, 2000). These facts led us to hypothesize that renal ischemia induces an increase in tissue nitric oxide levels likely coming from tissue nitrosothiol stores and therefore that this phenomenon should be dependent on the presence of thiol groups in the tissue. In a study performed in our laboratories (Salom et al, 2005) we found that renal ischemia is followed by a rapid increase in intrarenal nitric oxide concentration that is maintained until reperfusion, when a fast drop in nitric oxide levels near preischemic values is observed. The increased nitric oxide concentration observed seems to be independent of nitric oxide synthase and appears to originate in tissue nitric oxide stores that release nitric oxide during ischemia.

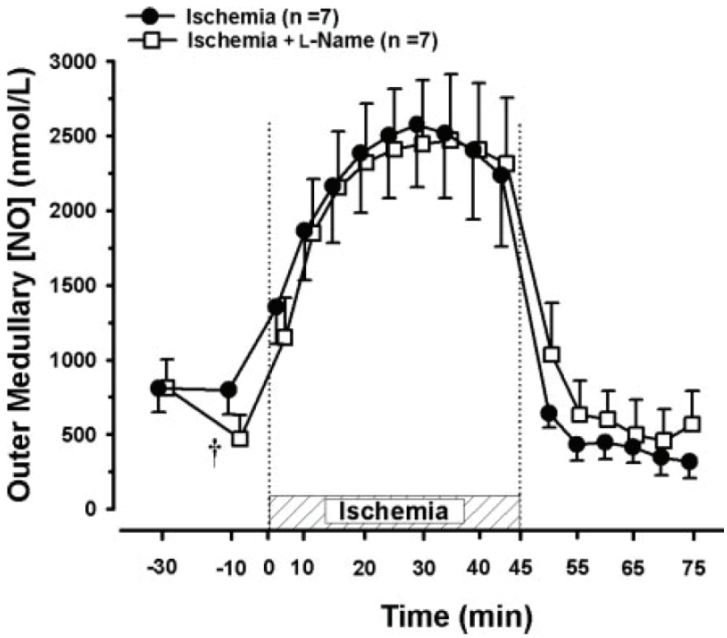

Fig. 2. Changes in nitric oxide levels in Outer Medulla during renal ischemia/reperfusion Changes in nitric oxide levels in outer medulla before, during and after a 45 min renal artery occlusion in SD rats infused with either saline (Ischemia) or L-Name (10 µg/kg/min, Ischemia + L-Name).).† Significant difference from the control period (-30 min) (Salom MG et al, 2005)

8. Sex differences in renal response to AKI

Females are known to suffer less severe renal I/R injury than males (Hutchens et al, 2008; Kang et al, 2004; Kher et al, 2005; Si et al, 2009; Wei et al, 2005; Xue et al, 2006) being the incidence of end stage renal disease approximately 50% higher in men than women. However, the mechanisms explaining this difference remain to be determined. It has been hypotethized that sex differences could be due to a higher renal constitutive nitric oxide synthase activity and/or increased nitric oxide bioavailability in females that protects the kidney against I/R injury (Chambliss & Shaul, 2002). However, lower, higher, or similar levels of endothelial and neuronal nitric oxide synthase expression in renal homogenates, cortex, and medulla of males and females have been reported (Erdely et al, 2003; Ji et al, 2005; Reckelhoff et al, 1998; Rodriguez et al, 2010; Wang et al, 2006; Wangensteen et al, 2004). However, the physiological meaning of nitric oxide synthase expression alone is uncertain, because dissociation between nitric oxide synthase expression and activity has been reported (Reckelhoff et al, 1998). The fact that nitric oxide availability depends not only on nitric oxide synthase expression and activity but also on the production of reactive oxygen species (because they inactivate nitric oxide in a concentration-dependent manner) also implies that sex differences could be due to a lower oxidative stress in females leading to increased nitric oxide levels (Arnal et al, 1996; Barbacanne et al, 1999) compared with

males (Brandes & Mügge, 1997). Thus, the reduced susceptibility of females to I/R injury may be due to a greater nitric oxide synthase activation, nitric oxide bioavailability, and/or lower free radicals formation during ischemia and early reperfusion, resulting in a less severe acute renal failure. In a recent study performed in our laboratory (Rodríguez et al 2010) we evaluated sex differences in outer medullary changes of nitric oxide and peroxynitrite levels during 45 min of ischemia and 60 min of reperfusion in SD rats. No sex differences were observed in endothelial and neuronal nitric oxide synthases nor in nitric oxide and peroxynitrite levels. We also found that a 45-min ischemia was followed after 24 h of reperfusion by a postischemic renal failure in males but not in females. This sex difference was associated with lower nitric oxide and greater peroxynitrite and 3-nitrotyrosine levels in males during ischemia, indicating increased oxidative and nitrosative stress. Pretreatment with the antioxidants N-acetyl-L-cysteine or ebselen abolished sex differences in peroxynitrite, nitrotyrosine, and glomerular filtration rate, suggesting that a greater oxidative and nitrosative stress worsens renal damage in males. Taken together, the data in the present study strongly suggest that the resistance of females to renal failure may be related to a greater renal tissular antioxidant capacity that could blunt the conversion of nitric oxide to peroxynitrite during ischemia.

9. Role of heme oxygenase system in renal I/R

Heme is a ubiquitous molecule with an active iron center with high affinity for oxygen which allows for transport of oxygen in hemoglobin and myoglobin. Heme also serves as the catalytic site in a variety of proteins involved in cell metabolism including respiratory chain cytochromes and numerous cytochrome P450 isoenzymes (Maines, 1997). Oxidative stress destabilizes heme proteins, leading to free heme release, which has prooxidant and toxic effects through free radical formation, and lipid peroxidation in renal tissues, (Akagi et al, 2002).

Intracellular free levels are tightly controlled in most cells and tissues by heme oxigenases (HO) which catalyze the initial and rate limiting step in heme catabolism (Tenhunen et al, 1968). Oxidative cleavage of heme molecules by HO yields equimolar quantities of biliverdin (BV), carbon monoxide (CO), and Fe^{+2}. Biliverdin undergoes further degradation to bilirubin (BR) by the cytosolic biliverdin reductase. All HO-derived products are biologically active substances: biliverdin and iron are believe to subserve antioxidant and prooxidant mechanisms, respectively (Abraham et al, 1997), whereas HO-derived CO exerts exerts vasorelaxant (Zhang et al, 2001), antiapoptotic, and anti-inflammatory effects.

Oxidative stress promotes the upregulation of the inducible isoform of heme oxygenase (HO-1) (Motterlini et al, 2002), which is expressed, along with the constitutive HO-2 isoform, in renal vascular and tubular structures in renal cortex and medulla (Abraham et al, 2009). HO-1 and HO-2 catalyze the same reaction and have similar cofactors requirements, but they differ with respect to the regulation and expression pattern. HO-2 accounts for the bulk of renal HO activity in normal conditions, whereas HO-1 operates as an inducible enzyme with low renal levels in the healthy kidney (Da Silva, 2001), but markedly increased in pathological conditions associated to hypoxia and inflammation (Otterbein et al, 2003).

Ischemia compromises organ function, which is further aggravated upon reperfusion, as a consequence of endothelial dysfunction, high levels of oxidative stress, altered renal

hemodynamic, and activation of the immune response. HO-1 prior to the induction of I/R results in functional protection in ischemic renal failure (Maines, 1999), which is partially mediated by reduction of oxidative and nitrosative stress during ischemia (Salom et al, 2007). Moreover, in the same way that HO metabolites maintain renal medullary perfusion (Zou et al, 2000), glomerular filtration rate and renal blood flow (Arregui B, 2004) in physiological conditions, HO-1 induction preserved postischemic medullary blood flow and GFR, in ischemic renal failure, (Salom et al, 2007). Overall, HO-1 induction might have multiple beneficial functions in I/R injury a) by reducing oxidative stress insults b) by preserving alteration of renal hemodynamic which contributes greatly to the subsequent renal failure and c) via suppression of the immune response through its anti-inflammatory actions, (Kotsch et al, 2007).

The molecular mechanisms underlying the protective role of HO induction in I/R injury are complex and likely multifactorial. Increased HO-1 activity in acute renal ischemia would result in the removal of the potent cell stressor heme (Akagi et al, 2005), but protection is also a consequence of the production of biologically active metabolites, i.e, CO, and BV. In this regard, the preadministration of exogenous CO donors in vivo have demonstrated functional protection comparable to HO-1 induction, pointing out to CO as a key component of the protection associated with HO-1 induction (Vera et al, 2005). Moreover, studies combining inhaled CO and infused bilirubin in rat renal transplantation demonstrated synergistic effects on glomerular filtration rate and renal blood flow (Nakao et al, 2005) suggesting also a role of bilirubin production in cytoprotection against ischemia/reperfusion injury. Finally, HO induction, through a variety of mechanisms, can reduce NO synthesis and, consequently, diminish augmented peroxynitrite formation during ischemia (Salom MG, 2007).

10. Concluding remarks, perspectives and significance

Ischemic injury to the renal vasculature may play an important role in the pathogenesis of both early and chronic ischemic acute kidney injury (AKI). Established and new data support the suggestion that vascular injury, in particular endothelial cell injury, participates in the extent and maintenance of AKI. Early alterations in peritubular capillary blood flow during reperfusion has been documented and associated with loss of normal endothelial cell function, which can be replaced pharmacologically or with cell replacement interventions. Distorted peritubular capillary morphology is associated with loss of barrier function that may contribute to early alterations in vascular stasis. In addition, ischemia induces alterations in endothelial cells that may promote inflammation and procoagulant activity, thus contributing to vascular congestion. Reductions in microvasculature density may play a critical part in the progression of chronic kidney disease following initial recovery from ischemia/reperfusion-induced AKI. The exact nature of how capillary loss alters renal function and predisposes renal disease is thought to be due at least in part to oxidative and nitrosative stress causing an endothelial balance between nitric oxide and peroxynitrite. Restoring the imbalance between nitric oxide and peroxynitrite will ameliorate endothelial dysfunction thus improving renal function. Finally, the loss of endothelial cell function may represent an important therapeutic target in which nitric oxide, vascular trophic support, and/or endothelial progenitor cells may show potential importance in ameliorating the acute and/or chronic effects of ischemic AKI. The use of drugs like statins that increase

hemo oxygenase-1 expression, and restores the normal imbalance beween nitric oxide and peroxynitrite (Heeba et al, 2009) and reduce postischemic renal failure (Gueler et al, 2002) seem to be promissory.

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12. References

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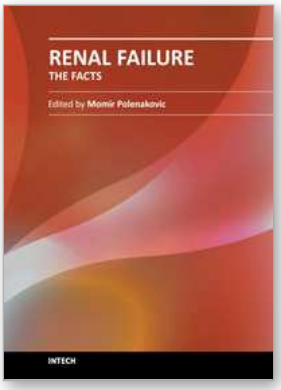

Renal Failure - The Facts

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The book "Renal Failure - The Facts" consists of some facts about diagnosis, etiopathogenis and treatment of acute and chronic renal failure. Acute, as well as chronic renal failure is great medical problems and their treatment is a burden for the budget of each government. The purpose of the chapters is to present some important issues of diagnosis and causes of AKI, as well as caused by snakes and arthropods, after cardiac surgery, as well as some therapeutic achievements in AKI. Well presented are the psychological condition in patients on haemodialysis, as well as the treatment of diabetic uremics. The book is aimed at clinicians with a special interest in nephrology, but it should also prove to be a valuable resource for any generalists who encounter a nephrological problems in their day-to-day practice.

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