IJNU Review Article

Int J Nephrol Urol, 2010; 2(1):157 - 171

Strategies to Extend Warm Ischemia Time during Laparoscopic Partial Nephrectomy- Part II

Thambi Dorai[1*], Catherine Michels[2], David S Goldfarb[3], Michael Grasso[1]

[1]Department of Urology, Saint Vincent Catholic Medical Centers, New York, USA

[2]Department of Urology, New York Medical College, Valhalla, USA

[3]Nephrology Section, New York Harbor Health Care System and New York University School of Medicine, New York, USA

Abstract

Background and Aims: In this part of the review, particular attention is drawn towards the mechanisms by which one can pre-condition the kidneys pre-operatively by using several strategies that are nutritional, biochemical, pharmacological and environmental in nature. In addition, ideas and hypotheses are put forward that are relevant to modulating the anoxic and autophagic response to ischemic damage during laparoscopic partial nephrectomy, particularly with respect to reducing the endoplasmic reticulum stress. Finally, the current trends in research involving the role of stem cells, renal hibernation and the role of the innate immune response are highlighted with the aim of exploiting these mechanisms to achieve reduced kidney damage during ischemia. Finally, the common thread behind all these approaches, namely empowering the renal mitochondria during ischemia is emphasized.

Keywords: Laparoscopic Partial Nephrectomy, Ischemia, Hypoxia, Anoxia, Mitochondrial Dysfunction, Reactive Oxygen Species

Some recent research trends in minimizing renal ischemic damage, relevant to LPN:

Exploiting the anoxic and autophagic response:

As explained in the first part of this review, the severe hypoxia developing in the affected kidney during laparoscopic partial nephrectomy (LPN) can quickly progress into total anoxia, depending upon the time it takes to perform the surgery. The response to anoxia is often called the unfolded protein response (UPR) (1). In tumor systems, hypoxia response is the way tumors respond to low oxygen tensions for their own survival and therefore hypoxia inducible factor-1 (HIF-1) inhibition is viewed as a direct target for therapy (2). However, a direct HIF-1 inhibition may not always inhibit tumor growth (3). In these situations, the anoxia response of these tumors may still be intact and functional and continue offer another level of protection that was not offered by hypoxia alone (4). Xenograft studies that knocked out various components of the anoxia

*Correspondence:
Thambi Dorai, Ph.D
Department of Urology, Cronin-666,
Saint Vincent Catholic Medical Centers,
170 West 12th Street, New York, NY 10011. USA.
Tel: 212-604-1556
Fax: 212-604-2073
E-mail: tdorai@svcmcny.org
Received: 25 Apr 2009
Revised: 20 May 2009
Accepted: 2 Jun 2009

response or the integrated stress response (ISR) such as ATF4, ATF6, XBP1 and PERK enzymes indicate a more profound inhibition of tumor growth indicating that the anoxic response is essential for tumor growth (4, 5). Alternatively, tumors may employ the anoxic response and adapt to zero concentrations of oxygen which may be more important than hypoxia alone for their survival. The other side of the coin in this issue is that, similar to the tumor situation where they may contain both hypoxic and anoxic components, the renal tissue during LPN (and/or in any other ischemic state due to disease) may also contain hypoxic and anoxic regions and may elicit both hypoxic and anoxic adaptation responses for its survival. Thus, while the expression of both these responses, which may have some common denominators as well as some unique features, may be cause to the progression of the tumor in a negative sense, these adaptive responses may be beneficial to LPN patients in a positive sense. Hence, a thorough knowledge of the hypoxic as well as the anoxic response of the tumors is absolutely essential since they can be extrapolated in reverse for the benefit of the patient undergoing LPN surgery or any other ischemia generating procedure. In this relation, activators of PERK kinase or agonists of ATF4 may be beneficial in initiating the anoxic stress response in the LPN patient before surgery which may offer some reno-protection. However, validation of these concepts will have to await further experimentation. The relationship between severe hypoxia bordering on anoxia and the tissue's response to cell injury due to endoplasmic reticulum (ER) and oxidative stress have been worked out well for the neuronal ischemic experimental model systems which showed that hypoxia activated the ER resident translational initiation factor eIF2α kinase and PERK kinase (6, 7). More recently, ER stress was shown to be a trigger for the induction of autophagy (8). These studies provide several molecular links between hypoxia, anoxia, the induction of apoptosis, necrosis and autophagy and provide the conceptual paradigm that depending upon the severity of the stress, the cell can either decide to go through survival or abort pathways of apoptosis or autophagy and/or necrosis (9). Extrapolated to the conditions that may exist in the kidney during LPN, depending upon the level of sudden oxidative and ER stress, hypoxia and anoxia, the cell may induce autophagy which is a survival response to low levels of autophagic stress. In this survival response, the injured cell may try to prevent necrosis through catabolic energy production and may prevent apoptosis via eliminating damaged mitochondria. However, a "high" level of autophagic stress may lead to massive lysosomal activation and cell demise as a consequence (10). It is the "low level" of autophagic stress that may protect the renal tissue against further oxidative damage during I/R by activating the survival mechanisms. Apart from information gleaned from the neural ischemia systems, studies by several groups using animal models for chronic myocardial ischemia also showed the induction of autophagy and the protective role (against apoptosis) played by this phenomenon (11). Again, at low levels of autophagic stress, these animal models re-emphasize the need to understand the role of the autophagic response as a survival response conferring ischemic stress resistance to the animal. This ischemic stress resistance, when applied after proper calibration, may offer a significant level of protection in the LPN patient. There are several studies supporting this notion, again in the chronically ischemic myocardium (CIM). It was shown that CIM often survives for years and recovers myocardial function after a coronary bypass surgery (12). This prolonged survival of CIM suggested the existence of cell protective mechanisms such as the activation of genes involved in protection from apoptosis, cell growth and cytoprotection such as IAP proteins, VEGF, Hsp70, HIF-1α, GLUT-1 etc during chronic ischemia in the myocardium which conferred cytoprotection (13). During a search for

additional mechanisms of cytoprotection, these investigators also found evidence for the induction of autophagy in CIM, suggesting that at a certain level this phenomenon could be cytoprotective too (14). Autophagy was also observed in recent studies in human myocardium due to chronic ischemia. Autophagy is a unique cellular degradation process responsible for the turnover of unnecessary and/or dysfunctional organelles and cytoplasmic proteins which become sequestered in a double membrane vesicles termed autophagosomes which subsequently degrade the proteins upon fusion with the resident lysosomes. There is growing evidence to show that autophagy could play an important protective role especially in the early stage of several diseases whereas extensive autophagy can cause a form of cell death, which is different from apoptosis suggesting that autophagy can in fact work like a double edged sword. These studies also revealed that at low level of stress, the function of autophagy is to prolong the life of a cell against micro environmental stresses such as nutrient starvation. When the nutrients are low, amino acids from degraded proteins can be re-used for functional recovery and help with the continued survival of the ischemic tissue. This process would also help to maintain the cellular ATP levels. However, when the ischemic insult is overwhelming so that these recovery processes may not be sufficient for survival, this protection from low autophagy may not be effective anymore and the cell may develop the autophagic type of cell death (15). In this respect, it is very interesting to note that Kim et al have recently found that autophagy plays a protective role in their model of chronic ischemia when induced by 6 episodes of intermittent ischemia/reperfusion (I/R) (16). This raises the paradigm that repetitive ischemic/reperfusion conditioning may offer cytoprotection not only to cardiac ischemia but also to other tissues such as the kidney in the LPN context. It may be possible to "prepare" the patient before LPN surgery with a defined number of short I/R episodes so that all the survival (hypoxic as well as the anoxic) mechanisms described above could be activated for the benefit of the LPN patient. This may very likely offer cytoprotection from the oxidative stress and other insults on the affected kidney during and after surgery. Indirectly, this type of pre-conditioning may also offer a way to prolong the warm ischemia time during the LPN procedure, during which it can be safely completed. However, further work is needed to figure out the exact number of episodes that can be safely and reproducibly used in man to maximize the ischemic pre-conditioning without having any detrimental effects. This type of reasoning may be relevant in the kidney transplantation context also.

Role of stem cells in minimizing the ischemic damage:

Epithelial cells of the renal tubule are particularly sensitive to injury during long ischemia time in LPN. This is mainly due to their poor oxygen supply but yet a higher energy demand for the transportation of several solutes in the glomerular filtration and re-absorption process. Several important transplantation studies have revealed that there is a mobilization of bone marrow cells into the kidney just after transplantation (17). This has led to the concept that certain bone marrow stem cells may be mobilized into the circulation, enter the kidney and may directly contribute to tubular repair and regeneration following ischemia. Further studies indicated that these mobilized cells could be bone marrow stromal cells, also called mesenchymal stem cells (MSCs) (18). In experimental models systems in mice with I/R injury or cisplatin nephrotoxicity, male donor cells were injected into female recipient mice and kidney sections were analyzed for Y-chromosome containing cells. Interestingly, it was found that only very few Y chromosome containing cells were able to populate the renal interstitium, primarily in areas surrounding the injured tubules (19). Moreover, a vast majority of cells that underwent proliferation

and repair were found to be endogenous tubular cells. These results indicated that the MSCs were, even though they are very few, are able to populate the injured area and secrete factors that might protect the injured cells from further damage and help them to proliferate, possibly through a paracrine effect. Following transient ischemia and reperfusion, the kidney is known to upregulate the expression of several factors that can mobilize bone marrow derived cells into the circulation. Notable examples of the secreted factors include stromal derived factor-1 (SDF-1) and interleukin-6 (IL-6) (20). This may help, at least in part, explain the journey of these MSCs to the kidney through a chemokine mediated attraction gradient. Further studies also indicated that there is a second population of mobilized cells from bone marrow that may be sensitive to SDF-1 and IL-6 that do not populate the renal parenchyma but appear instead to secrete several reno-protective factors (21, 22). In fact, in mice subjected to ischemic preconditioning of the heart, myocardial SDF-1 α mRNA was found to be increased 3 hours after ischemia. Moreover, the increase in SDF-1 mRNA also coincided with an increase in the SDF-1 as well as the cognate receptor protein CXCR4 in the experimental myocardium (23). These studies highly suggest that SDF-1 and CXCR4 may constitute a paracrine or autocrine axis in cardiac myocytes that is activated in response to ischemic pre-conditioning and hypoxic and/or anoxic stimuli. Moreover, these studies also revealed that, in response to these pre-conditioning stimuli, several anti-apoptotic kinases such as ERK and AKT were recruited and activated. This could promote an anti-apoptotic and survival program that confers protection against ischemia and reperfusion (I/R) damage. Very recent studies also indicated that this SDF-1/CXCR4 axis may also participate in the regeneration/repair processes by activating a neo-angiogenesis program (24). All these phenomena may very well occur in the case of LPN and other renal ischemic situations also. These impressive background studies suggest an important paradigm that a pre-conditional mobilization of these bone marrow derived stem cells (through stimulation from the direct administration of stem cell derived factors such as SDF-1) or a direct injection of (patient's own) purified bone marrow derived stem cells well before LPN surgery may offer reno-protection, repair and regeneration and hence may improve warm ischemia time due to this enhanced repair and potentiation capacity.

The concept of renal hibernation as a way to enhance warm ischemia time during LPN:

Entry into a state of metabolic rate depression or hypometabolic state is a survival strategy used by several organisms when challenged with environmental stress, including low or no oxygen, nutrients, cold temperatures *etc*. This capacity to survive and maintain long term viability and dormancy is conserved across several phyla. Several fish, snails and turtles that live deep in the hypoxic and cold waters have developed this hibernating capacity to survive several winter months. Ground squirrels, mole rats and hibernating polar bears are other good examples of adapting to hypometabolic state in order to survive the winter season. These animals have developed the capacity to reorganize their metabolic priorities such as ATP production and expenditure and to control other molecular processes to suppress functions such as transporters and protein synthesis etc which have a great ATP demand (25). They also have enhanced defense capacities to support the hypometabolic state and viability such as the expression of heat shock proteins, antioxidants and protease inhibitors. Thus, it is not surprising to see that humans also have developed several hypometabolic survival mechanisms which are only beginning to be discovered. For example, humans as well as other multicellular organisms have developed mechanisms to use both sulfur and oxygen as the electron acceptor in several reactions (26). Incidentally, sulfur

was the main electron acceptor in nature billions of years ago, when there was no oxygen. Even now, it is possible to locate biochemical reactions in man where free cysteine plays an important role in regulating oxygen utilizing enzymes *via* post-translational modifications (27, 28). This has led to the hypothesis that sulfide ion could play a critical role in buffering oxygen consumption. With this line of reasoning, several studies have shown that there was a reduction in oxygen consumption after exposure to hydrogen sulfide. Mice were pretreated with H_2S and then exposed to environments containing lethally low levels of oxygen. Control mice could not survive longer than 20 minutes when exposed to 5% oxygen. However, if mice were first subjected to a suspended animation state by a 20 minute pre-treatment with H_2S and then exposed to 5% oxygen, they could survive for more than 6.5 hours (29). It was concluded from these novel experiments that pre-treatment with H_2S reduces oxygen consumption and carbon dioxide production and forces the animals into a hypo-metabolic state which enables the mice to survive in lethally low oxygen supply. In another recent study, administration of H_2S at the time of reperfusion greatly limited the extent of myocardial infarction in an *in vivo* murine model (30). This myocardial cytoprotection was brought out essentially by a preservation of mitochondrial function. By titration analysis using increasing concentrations of H_2S it was concluded that a concentration of 10 μM H_2S administered during a 30 minute hypoxia was capable of reducing the mitochondrial oxygen consumption by 65% in a clear and reproducible manner and most importantly, this was followed by a complete recovery of the mitochondrial function to the baseline, after the H_2S was removed. These experiments were followed by observations that mitochondria isolated from mice given H_2S at the time of reperfusion displayed preserved mitochondrial function 24 hours post-reperfusion as analyzed by complex I and complex II efficiency. Electron microscopic analysis revealed a striking reduction in mitochondrial swelling and increased matrix density in mice receiving H_2S. This observation further suggested a prominent role of H_2S in preserving mitochondrial function in the observed cytoprotection. These critical experiments done on the ischemic myocardium may have direct relevance in the context of the kidney and this needs to be investigated in the future. It is highly possible that a prior conditioning/administration of H_2S to the LPN patient just before surgery may preserve the mitochondrial function. The unique way in which this preservation function is brought about is by decreasing the oxygen demand when there is a severely decreased oxygen supply. In many cases of kidney as well as myocardial ischemia /reperfusion (I/R) injuries, oxygen supply was greatly reduced (due to severe hypoxia and/or anoxia and ischemia) without any check on oxygen demand. Due to the inefficiency of the electron transport system in the mitochondria which normally misfires 1-2% of the time, this reduced oxygen supply with a huge oxygen demand is a perfect recipe to produce more reactive oxygen species (ROS). However, if the demand is suppressed (by H_2S) before a reduction in oxygen supply, then it is possible to infer that that less ROS would be generated and the ischemic damage to the cell would be avoided. Thus, in a scenario where the LPN patient receives a certain quantity of H_2S that is good enough for reducing the oxygen demand in the kidney in the first place just before surgery (without compromising the reversibility and recovery of mitochondrial function), a type of suspended animation state akin to hibernation can be produced on demand which may be expected to increase the survival times in lethal hypoxia without detrimental ROS mediated injury. By inference, this reasoning and strategy may be used to extend the warm ischemia time in LPN and for a successful completion of the procedure. However, these concepts should be investigated further (31). Apart from this, hepatocytes are also capable of anticipatory adaptation (hepatic hibernation) of

their metabolism that seeks to maintain cellular viability during prolonged and moderate hypoxia (32). Conceptually, even a switch from oxidative phosphorylation pathway to a glycolytic pathway to produce ATP (albeit in lower amounts) may be visualized as a form of anticipatory adaptation that would produce less ROS during kidney I/R or LPN. Several small molecule inhibitors of the oxidative pathway or activators of the glycolytic pathway are available but their therapeutic potential is not yet known. Incidentally and almost invariably, all cancers switch from oxidative form of mitochondrial metabolism to a glycolytic form of metabolism for utilizing glucose, even though the latter is 18 times less efficient as the former. Thus, the switch of all cancers to a glycolytic pathway can be viewed as an anticipatory adaptation pathway by which the cancer learns how to survive in low oxygen supply, reduce demand and at the same time have a survival advantage with less ROS production (33, 34). Thus, lessons learned from the glycolytic phenotype of tumors can be applied to extend the warm ischemia time during LPN by pre-conditioning or forcing the kidney to acquire the adaptation and survival capacity well before surgery that would produce less ROS and less complications subsequently.

Activation of the innate immunity responses before I/R:

Patients undergoing LPN surgery will inevitably undergo a certain level of ischemia/reperfusion (I/R) injury. This initial non-immune injury leads to the activation of an innate immune response causing variable degrees of tissue damage (35). The responses elicited by the ischemic kidney may be very similar to those elicited during or after renal transplantation. Thus, a pre-conditioning of the renal microenvironment before the LPN procedure or a modulation after the procedure may immensely benefit the affected tissue such that the procedure can be performed with a reasonable amount of extension (of warm ischemia) time without renal injury. The Toll Like Receptors (TLRs) are expressed not only by the cells of the immune system, but also by several tissues and cell types, including kidney tubular epithelial cells and mesangial cells and their expression increases in response to injury. Signaling *via* TLRs is dependent upon association with a group of cytoplasmic adapter molecules such as MyD88 and TRIF (36). Downstream effects of TLR engagement include production of inflammatory cytokines, chemokines and other soluble mediators that contribute to local inflammation and leukocyte accumulation which contributes to a robust adaptive immune response (37). In the event of sepsis, kidney expression of TLR4 is crucially involved in mediating LPS induced acute kidney failure *via* pro-inflammatory cytokine release and subsequent kidney damage (38). But, in the context of LPN, it is possible that certain endogenous ligands released from the hypoxic/anoxic kidney may signal through TLRs (39, 40). They include heat shock proteins released into the extracellular medium, high mobility group box 1 (HMGB1), hyaluranan, fibronectin *etc* (41, 42). Increasing experimental evidence indicates that engagement of the TLRs and the activation of the innate immunity responses by such endogenous ligands may be a major trigger for inflammation and other complications of ischemia (43-45). Also, these studies indicate that a deficiency of TLR2 (TLR2-/-) in mice protected mice from ischemic organ injury. Thus, it appears that TLR2 is constitutively expressed in the kidney and plays an important role in the pathogenesis of acute ischemic injury. Hence, there is an implication that TLR2 blockade could provide a basis of therapeutic strategies to treat or prevent renal ischemic injury. How and to what extent a renal TLR2 blockade can effectively and safely provide a protective function against I/R injury remains to be determined. But, it is reasonable to hypothesize that a pre-conditioning of the kidney before LPN surgery, possibly through a small molecule TLR (2

or 4) inhibitor or by a neutralizing antibody to TNF-α (or any other pro-inflammatory cytokine) and/or a neutralizing antibody to HMGB1 protein may tend to minimize the ischemic damage during surgery. This reno-protection strategy may open another avenue to extend the warm ischemia time during which this procedure can be safely completed.

Strategies that can be applied for reno-protection before the LPN procedure so as to extend the warm ischemia time without further damage:

In conclusion, this mini-review focused on several strategies to extend the warm ischemia time during LPN and to protect the kidney from oxidative and other inflammatory damages during and after LPN. The principles and hypotheses proposed here can be applied to other renal ischemic conditions and pathologies in a broad sense. There are also several recent reports that indicate the potential of several pre- procedure (pre-conditioning) strategies. We have grouped them as a) nutritional; b) biochemical; c) pharmacological and d) environmental for description purposes.

a) Nutritional:

Several nutritional supplements that are available over the counter to-day are proposed to reduce the renal ischemic damage in several conditions. Examples are grape seed extract, resveratrol, pomegranate juice, gooseberries, green tea, curcumin and vitamin-C (ascorbic acid). Most of these nutritional supplements may act through their ROS scavenging properties (46-49).

b) Biochemical:

In principle, any ROS scavenger when applied in a judicious way may be beneficial in the reno-protection process. Examples of ROS scavengers include glutathione, N-Acetylcysteine *etc* and administration of these substances may be beneficial. Injection of erythropoietin-1 (EPO-1) before LPN surgery to synthesize and mobilize more erythrocytes is another attractive possibility. Also, the reno-protection offered by EPO-1 may be partly due to its augmentation of HIF-1 α expression as described earlier. Administration of tin salts increases the expression of heme oxygenase-1 (HO-1) in a kidney specific manner (50). Tin salts, which have been thought of as simply toxic by earlier studies, may offer a new mode of treatment in the pre-conditioning of the kidney before LPN. Alternatively, injection of human recombinant stromal cell derived factor-1 (SDF-1) may engage the CXCR4 pathway in the kidney to activate the ERK/AKT survival and other renoprotective processes before LPN. Administration of citric acid cycle intermediates such as α-ketoglutarate with succinate or malate can bypass complex 1 in the mitochondria electron transport chain and produce ATP in the anerobic pathway and thus prevent the mitochondria from generating ROS from misfiring through complex 1 (51, 52). Thus, an injection of these citric acid cycle intermediates pre-operatively can offer some protection benefit in LPN and this may help in extending the warm ischemia time. Alternatively, direct injection of ATP and/or guanosine into the renal artery before ligation (during LPN) can be visualized as another approach (53). The bottom line here is that if we can replenish or supplement whatever nucleotides and other energy producing intermediates that are quickly depleted in the mitochondria as a result of severe hypoxia during LPN, the chances of full recovery without mitochondrial damage may be excellent. It is also very important to realize that renal ischemic insult involves not only sudden nucleotide depletion, but also sudden glucose deprivation. Thus, pre-operative supplementation of energy producing nucleotides and glucose as well as glutamine may be of therapeutic benefit. Thus, efforts may be taken just before LPN so that the kidney that is being operated upon is awash with all these reno-protective biochemicals. In a nutshell, empowering the mitochondria just

before LPN to withstand the ROS onslaught is the key to extend the warm ischemia time and also for a full recovery after the procedure. However, all these exciting possibilities need to be tested in the animal model system first, before they could be extrapolated to the human context. Another strategy is to treat the affected kidney with a manganese porphyrin derivative (MnPyP) as described earlier and reduce the renal injury *via* induction of key mitochondrial proteins such as complex V component ATP synthase, capable of blunting the oxidative injury (54). There are also reports that describe the protective function of nitrites (in a typical Mediterranean diet) due to their modulation of mitochondrial electron transfer at the level of complex I, particularly under ischemic conditions (55). It has been shown that nitrite can post-translationally modify Complex 1 by nitrosation. This process effectively dampens electron transfer through complex I, thereby reducing ROS production downstream. Thus, this type of nitrite pre-conditioning of the kidney prior to LPN may offer therapeutic benefits particularly in extending the warm ischemia time. Mannitol has been used by clinicians for the purpose of renal protection, particularly in the surgical patients about to undergo ischemic insult. However, there is no molecular mechanism that supports its use as compared to the strategies described above. As an alternative mechanism to alleviate the detrimental oxidative and inflammatory damages due to the liberation of pro-inflammatory cytokines, it may be worthwhile to consider the use of urinary trypsin inhibitor (UTI), apart from the more expensive neutralizing antibodies for molecules such as TNF- α and IL-1α (56). UTI in fact can neutralize several of the inflammatory processes due to the liberation of caspases and other proteases. Hence, a pre-treatment with UTI may be helpful for the prevention of I/R related injuries to the kidney during LPN.

c) Pharmacological:

Cyclosporine A (CsA) has been used extensively as an immunosuppressive agent in organ transplantation. But, recent studies have also implicated its potential in reducing I/R injury in kidneys of animal systems At 3mg/kg i.v., CsA significantly improved the functional, morphological and histological parameters of the kidney and attenuated the I/R related oxidative damages. This protective phenomenon could possibly be due to the fact that cyclosporine could bind and inhibit the heat shock chaperone protein that is an integral part of the mitochondrial permeability pore complex and prevent the release of cytochrome-c, other inflammatory and apoptotic signaling mediators (57). Thus, it is possible that a pharmacological pre-conditioning of the LPN patient with a low dose CsA may reduce the oxidative and inflammatory injury that may ensue. It is possible to extend the warm ischemia time without significant kidney damage as a result of renoprotection offered by CsA. However, this approach needs to be investigated further. It may also be essential to scavenge the reactive nitrogen species (RNS) apart from ROS that continue to be generated during I/R. In a recent study, chronic ebselen (which is a powerful scavenger of peroxynitrite) treatment decreased RNS levels, restored tetrahydrobiopterin and glutathione levels and decreased lipid peroxidation products. Moreover, this treatment also decreased the nitrosylated protein levels, which is a good indicator of I/R injury (58). As described earlier, it may also be possible to pharmacologically induce the expression of HIF-1α rapidly and transiently through the inhibition of specific prolyl hydroxylases (PHDs) just before, during and after the LPN surgery so that the induced HIF-1 α may render its renoprotective effects (59, 60). Our own work with hydralazine revealed that even though the HIF-1α protein was up-regulated in the pre-conditioned kidneys, the final effect was more negative due to a possible parallel up-regulation of the rennin angiotensin aldosterone system (RAAS) (60). These findings emphasize the

caution that must be exercised while interpreting the results of ischemic pre-conditioning (60). Earlier *in vitro* studies by other laboratories reported that kidney cells have a widespread capacity to activate the HIF system under systemic hypoxia, under regional hypoxia and in response to administration of PHD inhibitors. These studies have formed the basis for the concept that pre-conditional HIF-1α activation before the ischemic episode by pharmacological agents that may impart the ability to withstand the insult. Conferring this capacity on the kidney pre-operatively may, in theory, prolong the warm ischemia time during LPN. All these concepts need to be tested in clinical trials in the future. Apart from these agents, pharmacological agents such as lonidamine may also be considered for pre-conditioning the kidney before the ischemic insult during LPN. Lonidamine is currently under human clinical trials for its ability to inhibit aerobic glycolysis in cancer cells. Interestingly, it appears to enhance aerobic glycolysis in normal cells, through mechanisms very likely to involve a differential modulation of mitochondrial hexokinase (61).

d) Environmental:

There have been numerous investigations in sports medicine that highly suggest that altitude training can improve endurance in athletes, with the goal of improving performance at sea level. The accepted paradigm has been that the improved performance at sea level was primarily due to increased erythropoiesis and increased red cell mass due to reduced oxygen supply at altitudes. But, a closer look at the benefit offered by altitude hypoxia to the athlete include increased angiogenesis, glucose transport, glycolysis, pH regulation and an increased ability to tolerate lactic acid production. While all these improved parameters go to benefit the muscles of the athlete, there have not been many studies on how altitude hypoxia can benefit the kidney, while it possesses a well characterized capacity to up-regulate HIF-1α under reduced oxygen tensions (62). It is reasonable to assume that whatever benefit that is conferred on the muscle may also be true for the kidney since the concept of hypoxic pre-conditioning of the kidney to protect itself from ischemia/reperfusion (I/R) related damages is now well established. Altitude acclimatization at sea level, used by athletes, is well documented. In efforts to "bring the mountain" to the athlete, various hypoxia devices, hypoxia tents and nitrogen apartments have been devised (63). While this type of blood doping is illegal and/or unethical for the athlete, it may offer certain advantages to the LPN patient about to undergo surgery, since these devices can offer an environmentally mediated hypoxic pre-conditioning without the use of any chemicals and pharmacological agents as described above. These devices offer the patient to be acclimatized to hypoxia before the ischemic insult by repetitive and intermittent pre-conditioning through a cardiac as well as renal metabolic remodeling. Environmentally, it may also be possible to activate the HIF-1α targeted pathways by heat acclimation, a mechanism which works through the expression of heat shock proteins to achieve cross tolerance (64).

Secondly, it may also be possible to environmentally pre-condition the LPN patient by sulphides such as H_2S so that the demand for oxygen is inherently reduced, producing a reversible suspended animation state, as described earlier. This concept of thrifty expenditure of energy supplies by inducing a hypo-metabolic state in animals (and possibly in patients) by reorganizing the metabolic priorities is attracting increased attention, particularly after the development of H_2S donors such as IK-1001™ (65). In particular, this agent has been used to induce hypothermia and a hibernation state in animals that do not normally hibernate and is currently in Phase I trials. This animated state reduces the oxygen demand while at the same time conferring several protective functions to the kidney, including a reduced capacity to produce ROS. The prospect of

the use of IK-1001 before LPN is very exciting and worthy of trials, because of the reno-protection that it may offer. By inference, this reversible suspended animation strategy may enhance the warm ischemia time without compromising renal function. Other H_2S donors such as garlic are available naturally and can offer a potential nutritional benefit in the reduction of I/R mediated damages but more studies are certainly needed in this area (66).

Concluding Remarks:

Empowering renal mitochondria before LPN may be key to minimize I/R mediated damages and to prolong warm ischemia time:

As emphasized several times in this review, I/R injury involves a complex cascade of events, including loss of energy, loss of ionic hemostasis, production of reactive oxygen species and cell death. In this context, mitochondria emerge as the critical organelles mediating these events as they undergo major changes that may contribute to the injury that occur during I/R. Severe hypoxia and/or anoxia during LPN forces the renal mitochondria to re-prioritize its functions very quickly and hence enabling the mitochondria well before the surgery may be a preventive measure by which one can minimize the oxidative injury that may ensue during the procedure (67). It s hypothesized here that enabling the mitochondria to tolerate the ischemic insult (pre-emptively) i.e., pre-operatively may minimize the I/R injury and may extend the warm ischemia time. One example through which one can energize the mitochondria is through (dietary?) the administration of α-lipoic acid (LA). LA, also known as thioctic acid is a potent anti-oxidant that occurs naturally as a prosthetic group in various mitochondrial enzymatic complexes. The two sulfur molecules undergo cycles of oxidation and reduction, making LA capable of terminating damaging free radicals. Several beneficial properties have been attributed to LA such as a) specificity of free radical scavenging in both oxidized and reduced forms; b) interaction with other anti-oxidants; c) metal chelating activity; d) effects on gene expression; e) favorable bio-availability; f) location in both aqueous as well as membrane microenvironments and g) the ability to repair oxidative damage (68). Apart from free LA, nutritional supplements such as a mixture of acetyl-carnitine and LA are also commercially available (for example, Juvenon™). All these formulations are specifically designed with mitochondria in mind, making the case for helping the mitochondria at the time of its need (LPN, ischemia) (69). Apart from these mitochondria-friendly agents, mention should be made of the novel orally active anti-oxidant called MitoQ that has the ability to target mitochondrial dysfunction. This agent aims not only to mimic the role of the endogenous mitochondrial Co-enzyme Q10 (CoQ10) but also substantially enhances the anti-oxidant capacity of CoQ10 in a mitochondrial membrane potential dependent manner. This mitochondriotropic agent has the exciting capacity to deliver an anti-oxidant property to the intracellular organelle that is responsible for increased levels of potentially damaging ROS (70). Potential use of agents such as MitoQ which are still under clinical development, prior to LPN surgery may enable the mitochondria to generate less ROS and to tolerate damage, effectively enhancing the warm ischemia time. All the potential reno-protective strategies discussed in this review are diagrammatically described in Figure 1. It may also be noted here that if the LPN patient is already on medications such as statins which seriously interfere with the synthesis of ubiquinones (CoQ10) in the first place (through their capacity to inhibit HMG CoA reductase), his/her mitochondrial function may already be compromised before LPN and this may have a bearing on how well the patient can tolerate ischemia during the procedure (71). As a consequence, the length of warm ischemia time that is tolerated by the patient without compromising renal function may be an

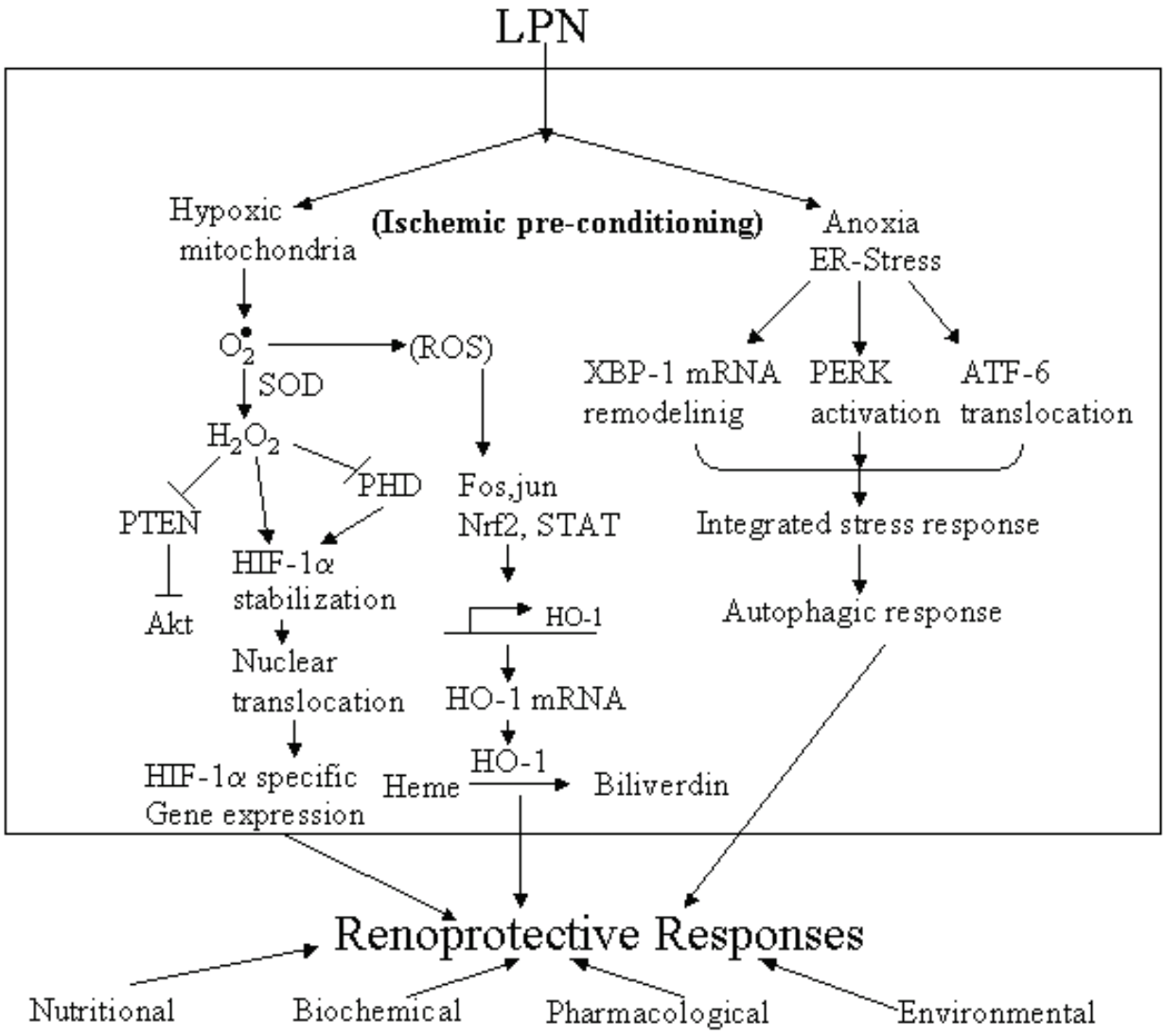

Figure 1. An overall view of the strategies that are proposed to minimize the ischemic insult and to enhance warm ischemia time during LPN without compromising on renal function. It is proposed that a suitable pre-operative ischemic pre-conditioning strategy needs to be employed that is targeted toward empowering the mitochondria to tolerate severe hypoxia and/or empowering the endoplasmic reticulum (ER) to tolerate ER stress during anoxia so as to exhibit an integrated stress response (ISR). Current progress in the field highly suggest that both these hypoxic and anoxic pre-conditioning strategies, when judiciously employed before LPN may provoke a broad-spectrum reno-protective response and pre-condition the patient to tolerate the ischemic insult that ensues during the procedure. It is hypothesized that this may translate into a longer warm ischemia time that is tolerated by the patient. Several of these approaches that may elicit these reno-protective responses are classified into nutritional, biochemical, pharmacological and environmental strategies which are described in this review.

inverse function of statin use.

In this review, we have made an attempt to describe the molecular mechanisms that underlie ischemic damage suffered by the kidney possibly through LPN and other pathologic conditions and to offer ways to minimize this damage so as to extend the warm ischemia time without compromising renal function. It is our hope that the strategies and hypotheses proposed here would stimulate further discussion and research that can be translated from the bench to bedside.

Acknowledgment

This work was supported in part by the Susan Schott Memorial Fund and the Department of Urology, Saint Vincent Medical Center.

Conflict of Interest

None declared.

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