Devil’s Triangle in Kidney Diseases: Oxidative Stress, Mediators, and Inflammation

Guest Editors: Ayşe Balat, Halima Resic, Guido Bellinghieri, and Ali Anarat
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Contents

Devil's Triangle in Kidney Diseases: Oxidative Stress, Mediators, and Inflammation, Ayşe Balat, Halima Resic, Guido Bellinghieri, and Ali Anarat
Volume 2012, Article ID 156286, 2 pages

Urotensin-II: More Than a Mediator for Kidney, Ayşe Balat and Mithat Büyükçelik
Volume 2012, Article ID 249790, 7 pages

Antioxidants in Kidney Diseases: The Impact of Bardoxolone Methyl, Jorge Rojas-Rivera, Alberto Ortiz, and Jesus Egido
Volume 2012, Article ID 321714, 11 pages

Induction of Oxidative Stress in Kidney, Emin Ozbek
Volume 2012, Article ID 465897, 9 pages

Inflammation and Oxidative Stress in Obesity-Related Glomerulopathy, Jinhua Tang, Haidong Yan, and Shougang Zhuang
Volume 2012, Article ID 608397, 11 pages

Reactive Oxygen Species Modulation of Na/K-ATPase Regulates Fibrosis and Renal Proximal Tubular Sodium Handling, Jiang Liu, David J. Kennedy, Yanling Yan, and Joseph I. Shapiro
Volume 2012, Article ID 381320, 14 pages
Editorial

Devil’s Triangle in Kidney Diseases: Oxidative Stress, Mediators, and Inflammation

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Received 13 November 2012; Accepted 13 November 2012

This issue of the International Journal of Nephrology focused on kidney diseases within a devil’s triangle, oxidative stress (OS), mediators, inflammation, specifically relating to the clinical significance of identification, and prevention.

Every creature in need of oxygen faces OS. It has a critical role in the molecular mechanisms of renal injury in several kidney diseases, and many complications of these diseases are mediated by OS, mediators, and inflammation. There is a complex relationship between these three; mostly they induce each other. While some of the diseases themselves can contribute to OS, reactive oxygen species (ROS) produced by activated leukocytes and endothelial cells in sites of inflammation cause tissue damage. Although inflammation looks dangerous for the organism, it is a normal reaction of organs and tissues to protect themselves against several invasion(s). It enables the immune system to remove the injurious stimuli and initiate the healing process of tissues. However, the interactions between OS, mediators, and inflammation may result in glomerular damage, proteinuria, electrolyte, and volume instabilities which cause nephron loss, on the long view. Detailed studies on this topic are included in this issue.

The kidney can easily be damaged by ROS, due to the rich structure of long-chain polyunsaturated fatty acids. The article by E. Özbek summarizes the induction of OS within kidney in several conditions, including diabetes, hypertension, hypercholesterolemia, obesity, aging, urinary obstruction, environmental toxins, and molecular mechanisms of these inductions in the light of existing literature data.

Diabetic nephropathy is one of the most common microvascular complications of type 1 and type 2 diabetes mellitus and the leading cause of end-stage renal disease worldwide [1].

Rojas-Rivera et al. reviewed the biological bases of oxidative stress and its role especially on diabetic nephropathy, as well as the role of the Keap1-Nrf2 pathway, and recent clinical trials targeting this pathway with bardoxolone methyl, a novel synthetic triterpenoid with antioxidant and anti-inflammatory properties.

Obesity continues to be a public health problem throughout the world. Epidemiologic studies have shown that 66% of adults and 16% of children and adolescents are overweight or obese [2]. Obesity-related glomerulopathy is an increasing cause of end-stage renal diseases. J. Tang et al. stressed the chronic low-grade systemic inflammation in obesity and discussed the roles of inflammation and oxidative stress in the progression of obesity-related glomerulopathy and possible treatment modalities to prevent kidney injury in obesity, such as the usage of anti-IL-6 receptor antibody, TNF-α antagonist, adiponectin, nutritional and surgical interventions to reduce OS.

Hypertension is another important global health issue both in adults and children. It is one of the major risk factors for the progression of kidney diseases. The relationship between blood pressure and dietary sodium and salt sensitivity has been well known, and renal sodium handling is a key determinant of long-term blood pressure regulation [3]. There is a limited knowledge in the literature regarding the role of ROS-mediated fibrosis and renal
proximal tubule sodium reabsorption through the Na/K-ATPase. S. Liu et al. reviewed the possible role of ROS in the regulation of Na/K-ATPase activity. The authors emphasized the importance of further researches whether ROS signaling is a link between the Na/K-ATPase/c-Src cascade and NHE3 regulation and how OS, stimulated by high salt and cardiotonic steroids, regulates Na/K-ATPase/c-Src signaling in renal sodium handling and fibrosis.

Urotensin-II is the most potent mammalian vasoconstrictor identified to date, almost tenfold more potent than endothelin-I [4]. A. Balat and M. Büyükçelik discussed the role of urotensin-II on renal hemodynamics and its possible role on several kidney diseases, such as the minimal change nephrotic syndrome. The article includes a detailed discussion of urotensin-II immunoreactivity in renal biopsy specimens of children with membranoproliferative glomerulonephritis, membranous nephropathy, IgA nephropathy, Henoch-Schönlein nephritis, and focal segmental glomerulosclerosis. Because of its complex relation with OS and other mediators, authors describe it as “more than a mediator” in glomerular diseases. They briefly mention from the effectiveness of U-II antagonism, as a new promising pharmacological treatment target in some kidney diseases.

Given the potential impact of OS, mediators, and inflammation trio, the importance of prevention has come into question. Strong evidence indicates the importance of new molecules that are able to diminish them which in turn may help to decrease the prevalence and/or progression of several kidney diseases. Therefore, further researches are needed to the better understanding of the molecular and clinical mechanisms of this triad. They may help to provide new therapeutical strategies to control several complications in patients with kidney diseases.

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References

Review Article

Urotensin-II: More Than a Mediator for Kidney

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Received 30 November 2011; Accepted 6 September 2012

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Human urotensin-II (hU-II) is one of the most potent vasoconstrictors in mammals. Although both hU-II and its receptor, GPR14, are detected in several tissues, kidney is a major source of U-II in humans. Recent studies suggest that U-II may have a possible autocrine/paracrine functions in kidney and may be an important target molecule in studying renal pathophysiology. It has several effects on tubular transport and probably has active role in renal hemodynamics. Although it is an important peptide in renal physiology, certain diseases, such as hypertension and glomerulonephritis, may alter the expression of U-II. As might be expected, oxidative stress, mediators, and inflammation are like a devil’s triangle in kidney diseases, mostly they induce each other. Since there is a complex relationship between U-II and oxidative stress, and other mediators, such as transforming growth factor β1 and angiotensin II, U-II is more than a mediator in glomerular diseases. Although it is an ancient peptide, known for 31 years, it looks like that U-II will continue to give new messages as well as raising more questions as research on it increases. In this paper, we mainly discuss the possible role of U-II on renal physiology and its effect on kidney diseases.

1. Introduction

Although urotensin-II was firstly identified in a neurohemal organ of fish in 1980s [1, 2], only recently it became a major focus of clinical and experimental researches [3].

Human urotensin-II (hU-II) is a cyclic peptide of 11 amino acids cleaved from a larger prepro-U-II precursor peptide of about 130 amino acids [1, 3, 4]. The gene encoding this peptide is located at 1p36 and contains 5 exons [5]. It is a ligand for the orphan G-protein-coupled receptor, known as GPR14 [3, 6, 7].

Although human prepro-U-II mRNA is expressed mainly in the brain and spinal cord [4, 8], both hU-II and its receptor are also detected in other organs and tissues, such as kidney, spleen, smooth muscle, endothelium, small intestine, thymus, prostate, pituitary, and adrenal gland [1, 9–11].

Being almost tenfold more potent than endothelin-I (ET), it is the most potent mammalian vasoconstrictor identified to date [3, 12]. It circulates in the plasma of healthy individuals and acts as a circulating vasoactive hormone and as a locally acting paracrine or autocrine factor in cardiovascular regulation [4, 13].

Although it mainly has a vasoconstrictor effect, regional differences may be seen in its effects in various vascular beds and blood vessels of some species. For example, it has a vasodilatory effect on the small arteries of rats [14, 15], and on the resistance arteries of humans, through release of endothelium-derived hyperpolarizing factor (EDHF), nitric oxide (NO) [14–16].

It has been shown that the potent vasoconstrictor actions of U-II are mediated by Ca²⁺ mobilization through activation of a number of signaling pathways including Ca²⁺ channels, tyrosine kinase, p38 mitogen-activated protein kinase (p38MAPK), and extracellular signal-regulated kinase 1 and 2 (ERK1/2) [17, 18].

Since U-II and its receptor have been demonstrated in mouse, monkey [19], and human kidneys [20, 21], it is acceptable to consider that U-II is synthesized, secreted, and cleared by the kidneys [6, 22–24].

Interestingly, Mosenkis et al. [25] showed that hU-II was also present in 2 surgically anephric subjects. Although this finding is inconsistent with the conclusion that the kidneys are the primary source for production of U-II, as the authors stressed, the high density of U-II and its receptor in renal
tissues suggest that U-II is metabolically active in the kidney even though it is produced outside of the kidneys.

In this paper, we mainly discuss the possible role of U-II on renal physiology and its effect on kidney diseases.

2. Effect of U-II on Renal Hemodynamics

Because of its potent vasoconstrictor effect, U-II attracted the interest of researchers in general hemodynamics. In fact, hemodynamic responses to U-II show regional heterogeneity in relation to its receptor localization, even in the differences of functional state of the endothelium [26].

However, in contrast to animal studies, Wilkinson et al. found no vasoactive responses to hU-II in vivo in man [27]. They injected hU-II intra-arterially to healthy male volunteers, and despite the high-circulating hU-II levels, no change was seen in systemic hemodynamics, ECGs of subjects, and hU-II had no effect on hand vein diameter.

However, another study published in the same year [28] demonstrated that U-II produces potent vasoconstriction in humans in vivo. They showed that U-II induced dose-dependent reduction in forearm blood flow (FBF) of healthy volunteers, and FBF returned to baseline values within 30 min.

Known data in the literature show that the kidney is a major source of U-II in humans [29], primates, mice [19], and rats [11]. It has been found in the urine of humans [22, 24] and rats [11] at a concentration far exceeding that of plasma. In humans the renal clearance of U-II has found to be greater than that of creatinine, suggesting that urinary U-II is derived primarily from the kidney [22]. An animal study also revealed that there was an arteriovenous concentration gradient for U-II across the renal circulation in anaesthetised sheep [30]. As well as U-II, its receptor has also been localized to the mammalian kidneys, such as in human [22], monkey, mouse [19], and rat kidneys [11]. It has been shown that the medulla, especially tubular component of the kidney, is the principal site of U-II receptor expression in the rat kidney [11, 31].

Shenouda et al. [20] demonstrated that U-II was mostly present in the epithelial cells of tubules and ducts, with greater intensity in the distal convoluted tubules in normal human kidneys. Moderate U-II immunoreactivity was seen in the endothelial cells of renal capillaries, but only focal immunoreactivity was found in the endothelial cells of the glomeruli. We also observed similar results in kidneys of children [21], and these findings suggest that hU-II may contribute to the pathophysiology of human kidneys (Figure 1(a)) [21, 32].

Expression of U-II mostly in tubules may suggest its probable active role in renal hemodynamics. Lorentz and Bern [33] demonstrated that U-II stimulated Na transport in the teleost urinary bladder, while Ovcharenko et al.’s study [34] indicated that short-term administration of U-II did not influence sodium (Na) handling by the kidney in rats. However, Zhang et al. [35] observed that infusion of U-II directly into the rat renal artery increased renal blood flow (RBF), associated with a diuresis and natriuresis. In contrast, Song et al. [11] reported that U-II caused an antinatriuresis and antidiuresis when administered as an i.v. bolus dose and stressed that this was associated with and driven by renal hemodynamic effects leading to a marked reduction in glomerular filtration rate (GFR).

Two years later from the above study, Abdel-Razik et al. [36] searched the potential direct tubular action of urotensin in rats. They observed dose-dependent changes in GFR and urinary electrolytes. The hemodynamic effects were predominated at higher doses and caused a profound reduction in GFR which was accompanied by an antidiuresis and anti-natriuresis. When a lower infusion rate of rat U-II was employed, a tubular action to reduce electrolyte reabsorption became apparent through an increase in fractional excretion of Na and potassium (K) [36].

However, in children with minimal change nephrotic syndrome (MCNS), and their healthy controls, we could not find any relationship between the U-II level and Na/K excretion [24]. Although this differences may be partially related to different biological effects of U-II in different species, contradictory observations in same species underline the complex influence of U-II on renal hemodynamics.

It is not clear enough whether the effect of U-II on tubular transport is direct or mediated by secondary mechanisms. However, considering the expression of U-II receptor in the thin ascending limb of Henle's loop and the inner medullary collecting duct [11] and the greater U-II receptor mRNA and protein expression in the medulla compared with the cortex [36], together with the abundant expression of hU-II in the proximal and distal tubules of children (patients and controls) [21], it may be suggested that U-II may have a direct action on tubular electrolyte transport.

3. The Effect of Urotensin-II on Kidney Diseases

Since U-II and its receptor, GPR14, are expressed abundantly in cardioenal system [10, 11], most of the researchs on it are related to cardiovascular and renal diseases.

Although some studies have been investigated the circulating levels of U-II in several diseases, such as hypertension [37], congestive heart failure [38], renal failure [3], MCNS [24], and preeclampsia-eclampsia [39], little is known about the actions of this important peptide within the kidney. Some studies suggest that renal dysfunction affects the U-II levels, since the plasma U-II level has been found elevated in renal failure [3], congestive heart failure [38], and systemic hypertension [37], and it was found to be an inverse predictor of overall and cardiovascular mortality in patients with moderate-to-severe chronic kidney disease (CKD) [40].

Certain diseases, such as hypertension and glomerulonephritis, may alter the expression of U-II. It has been shown that both U-II and its receptor mRNA expression levels were up to threefold higher in spontaneously hypertensive rat (SHR) tissue compared to control Wistar-Kyoto (WKY) rats, taking into consideration that SHR is more sensitive than WKY to the effect of U-II [41].
In the literature, there are no enough data on the level of this vasoactive peptide in glomerular diseases. Recently, we firstly demonstrated that U-II was present in plasma and urine samples of 26 children with MCNS [24]. It showed important changes in relapse and remission periods. Plasma U-II concentrations during relapse were significantly lower than in remission and in controls, whereas urinary U-II levels were higher in relapse than in remission [24]. The plasma U-II level showed a significant positive correlation with the plasma albumin concentration during remission. However, there was no correlation between the amount of proteinuria and plasma/urinary U-II levels, and we could not detect any relationship between U-II levels and other clinical and laboratory parameters (such as the age at onset of disease, number of relapses, time to remission, blood pressure, serum creatinine, and hematological parameters). We suggested that the important changes in plasma and urinary U-II levels during relapse may be the result of heavy proteinuria rather than playing a role in mediating the clinical and laboratory manifestations of MCNS. After this, it would be interesting to search the possible role(s) of this peptide in children with glomerular diseases other than MCNS. Therefore, we examined the urotensin-II immunoreactivity in renal biopsy specimens of children with several renal diseases, including membranoproliferative glomerulonephritis (MPGN), membranous nephropathy (MGN), IgA nephropathy (IgAN), Henoch-Schönlein nephritis (HSN), and focal segmental glomerulosclerosis (FSGS) [21, 32]. In normal human kidney, there was weak expression of human U-II in glomerulus, while abundant expressions were seen in proximal, distal tubules, and collecting ducts (Figure 1(a)) [21], similar to a previous study [20].

We observed different expression pattern of U-II in different glomerular diseases. In MPGN and FSGS, different from the normal kidneys, more dense U-II immunoreactivity was seen in the glomerular basement membrane (GBM), glomerular mesangium, Bowman capsule (BC), and tubules (Figures 1(b), and 1(d)) [21, 32]. Interestingly, we also observed U-II immunoreactivity in crescents (Figure 1(c)), and sclerotic areas in FSGS (Figure 1(d)) [21, 32].

Systolic blood pressure (BP) was positively correlated with mesangial expression of U-II \((r = 0.418, P = 0.042)\), while diastolic BP was correlated with endothelial U-II expression in MPGN \((r = 0.469, P = 0.021)\) [21].

In children with MGN, U-II was mostly seen in GBM and BC. We observed more dense U-II immunoreactivity in distal tubules \((P = 0.030)\), endothelium \((P = 0.009)\), and mesangium \((P = 0.002)\) in children with MPGN than in MGN. Diastolic BP was positively correlated with the expression of U-II in BC in children with MGN \((r = 1, P = 0.000)\) [21].

There is no enough data about the precise role of hU-II in renal diseases, and that was the first report demonstrating the presence of U-II by immunohistochemically in children with...
several renal diseases, suggesting that hU-II may contribute to the pathophysiology of human kidneys.

The positive correlation between BP and intensity of U-II expression in mesangium and endothelium in MPGN, and BC in MGN was noteworthy. Considering the literature data about U-II, as an important physiological mediator of vascular tone and blood pressure in humans [16], and also an extremely potent constrictor of renal blood vessels from primates [6], it is reasonable to suggest that U-II may play an important role in the regulation of BP in MPGN and MGN.

As it has been known, mainly, two basic mechanisms are feasible in glomerulonephritis: antibody interaction with antigens in situ within the glomerulus and antibody binding to soluble antigens in the circulation, followed by immune-complex deposition within the glomeruli [42]. The secondary immune mechanisms of glomerular injury are the cascade of inflammatory mediators that are recruited to propagate renal damage following the primary glomerular attack. Some of these mediators play essential roles, whereas others may aggravate the glomerular lesion [42]. Most of the secondary mediators include cytokines, growth factors, reactive oxygen metabolites, bioactive lipids (platelet-activating factor and eicosanoids), proteases, and vasoactive substances, such as ET and NO [42].

Since U-II is abundantly expressed in the glomeruli in MPGN and MGN, it is reasonable to suggest that U-II may play a role in this mechanism, probably in the secondary immune mechanisms of glomerular injury, by a paracrine or endocrine action [21]. Djordjevic et al. [43] demonstrated that hU-II increases the levels of NADPH oxidase-derived reactive oxygen species, leading to the activation of mitogen-activated protein kinases and protein kinase B (akt), followed by enhanced plasminogen activator inhibitor-1 expression and increased proliferation of pulmonary arterial smooth muscle cells. It has been also shown that exposure of the rat proximal tubular epithelial cells (NRK-52E) to transforming growth factor β1 (TGF-β1) or angiotensin II (Ang II) increased U-II and GPR14 mRNA expressions [44], and U-II acts synergistically with Ang II [45, 46]. As might be expected, oxidative stress, mediators, and inflammation are like a devil's triangle in kidney diseases, mostly they induce each other. Since there is a complex relationship between U-II and oxidative stress [43], and other mediators, such as TGF-β1 and Ang II [44–46], U-II is probably more than a mediator in glomerular diseases and takes place in an important part of this devil's triangle.

Interestingly, we observed abundant U-II immunoreactivity in crescents and sclerotic areas in FSGS [21, 32]. Crescents are composed of large swollen cells arising from both macrophages of hemogenous origin and native parietal epithelial cells [47]. As time elapses, the cellular crescents are progressively replaced by fibroblasts, and in more advanced stages, the fibroblastic component is entirely replaced by collagenous lamellar materials with a few remnant cells [48]. Recent reports have shown a mitogenic role for U-II through induction of smooth muscle cell proliferation [49, 50], and additionally, it has been shown to induce collagen deposition by fibroblasts [51]. Zhang et al. [52] showed that U-II could stimulate the phenotypic conversion, migration, and collagen synthesis in adventitial fibroblasts. Additionally, it may act as autocrine/paracrine growth stimulators in tumor cells [53].

The pathogenesis of glomerulosclerosis is still unknown. Several factors, cytokines and growth factors, hyperlipidemia and platelet activation, lead to an increase of mesangial matrix production by resident cells. Several data demonstrate that abnormal glomerular growth is associated with glomerular sclerosis [54].

Since hU-II stimulates cell proliferation in adrenal tumors [55], renal epithelial cells [23], and vascular smooth muscle cells [49] and it has been found elevated in carotid and aortic atherosclerotic plaques [56], the abundant expression of U-II in crescents and sclerotic areas suggests that U-II may also play a role in the progression of crescents and glomerular sclerosis, probably as a growth factor or as an inflammatory peptide. This hypothesis must be searched and tested in future.

MGN is an antibody-mediated disease of uncertain and imprecise pathogenesis. However, the hypotheses that it is an autoimmune disease of the kidney and that the subepithelial immune deposits are formed in situ with an endogenous glomerular antigen are attractive [57]. The electron-dense deposits are generally located at the site of the slit diaphragm, and subepithelial space, while no electron-dense deposits are seen in the subendothelial space or in the mesangium, and hypertension at onset is associated with a less favorable outcome in MGN [57]. In our study, hU-II expression was mostly seen in GBM and BC, and there was a strong positive correlation between diastolic BP and intensity of U-II expression in BC. These findings may increase two interesting questions: may U-II play a role in the formation of these deposits as a mitogenic factor, as we mentioned previously, and may it have any role in the clinical course of MGN by regulating the BP? However, it is difficult to answer these questions with that study, and these hypothesis must be clarified by further detailed studies.

In kidneys of children with HSN and IgAN, similar to each other, more dense U-II immunoreactivity was seen in GBM, glomerular mesangium, BC, proximal/distal tubules, and also in crescents [21, 32].

Although the pathogenesis of IgAN and HSN is not well known [58], animal studies have shown the key role of cytokines and growth factors (particularly platelet-derived growth factor and TGF-β) in the induction and resolution of mesangial injury, and there is some evidence that these are also involved in IgAN [58]. The similar expression pattern of U-II in HSN and IgAN has been considered that U-II may have a role in mesangial inflammation and crescent formation in these disorders [32].

Different expression pattern of U-II in several renal diseases may give rise to thought whether the effect of U-II gene polymorphism. Recently, we performed a preliminary study, and firstly investigated the possible association between a coding single nucleotide polymorphism of UT-II gene, T21M (T/C), in 87 children with childhood nephrotic syndrome (NS), 16 children with acute poststreptococcal glomerulonephritis (APSGN), and 10 children with HSN [59]. We found higher TC genotype of U-II gene in NS
(56.3% versus 38.9%, \( P = 0.025 \)), higher TT genotype in APSGN (50.0% versus 25.9%, \( P < 0.001 \), and a positive correlation between TT polymorphism and the presence of macroscopic hematuria in APSGN \( (r = 0.51, P = 0.04) \). This study considered that urotensin-II may be an important mediator in pathophysiology of the childhood glomerulonephritis, and Turkish children with TC genotype may have a higher genetic susceptibility to NS, while TT genotype of U-II may increase the risk of APSGN.

4. Urotensin-II Antagonists as a New Promising Pharmacological Treatment Target

Several influences of U-II in cardiovascular/renal system, and the presence of its receptor in the heart, lungs, blood vessels, kidneys, and brain, led the researchers to investigate the role of U-II antagonists in various diseases. The most known U-II receptor antagonists are palosuran and urantide. Sidharta et al. [60] investigated whether palosuran, a potent, selective, and competitive antagonist of the U-II receptor, had effects in macroalbuminuric, diabetic patients who are prone to the development of renal disease. They observed an overall clinically significant reduction of 24.3% in the 24-hour urinary albumin excretion rate.

In an experimental study, it has been shown that long-term treatment of streptozotocin-induced diabetic rats with palosuran improved survival, increased insulin, and slowed the increase in glyceria, glycosylated hemoglobin, and serum lipids. Furthermore, palosuran increased renal blood flow and delayed the development of proteinuria and renal damage [61].

These two researches suggest that U-II receptor antagonism might be a new therapeutic approach for the treatment and/or prevention of diabetic nephropathy. The tolerability and safety, pharmacokinetics, and pharmacodynamics of palosuran were evaluated in also healthy young men with a double-blinded placebo-controlled single ascending dose designed study [62]. It has been shown that palosuran was well tolerated, and no serious adverse events or dose-related adverse events were reported. However, as the authors stressed, the results of this entry-into-humans study warrant further investigation of the therapeutic potential of palosuran.

Recently, Nitescu et al. [63] examined the effects of another selective U-II receptor antagonist, urantide, on renal hemodynamics, oxygenation, and function in endotoxemic rats. However, they found that urantide had no statistically significant effects on any of the investigated variables (kidney function, renal blood flow, cortical and outer medullary perfusion, and oxygen tension) in these rats.

In spite of different results about the effectiveness of U-II antagonism, it appears that the therapeutic potential of U-II antagonists may be the focus of research interest in the near future.

In summary, U-II may be an important mediator, in fact probably more than a mediator, in kidney diseases. Whether the observed findings which are primary or secondary to these pathological conditions still remain unclear, they suggest a possible role of U-II in the pathophysiology of several kidney diseases. It looks like that U-II will continue to give new messages as well as raising more questions as research on it increases. Further, detailed studies are needed to address the exact role(s) of this peptide in renal diseases.

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Antioxidants in Kidney Diseases: The Impact of Bardoxolone Methyl

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Received 15 September 2011; Revised 2 April 2012; Accepted 10 April 2012

Academic Editor: Ali Anarat

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Drugs targeting the renin-angiotensin-aldosterone system (RAAS) are the mainstay of therapy to retard the progression of proteinuric chronic kidney disease (CKD) such as diabetic nephropathy. However, diabetic nephropathy is still the first cause of end-stage renal disease. New drugs targeted to the pathogenesis and mechanisms of progression of these diseases beyond RAAS inhibition are needed. There is solid experimental evidence of a key role of oxidative stress and its interrelation with inflammation on renal damage. However, randomized and well-powered trials on these agents in CKD are scarce. We now review the biological bases of oxidative stress and its role in kidney diseases, with focus on diabetic nephropathy, as well as the role of the Keap1-Nrf2 pathway and recent clinical trials targeting this pathway with bardoxolone methyl.

1. Background

Chronic kidney disease (CKD) is a serious public health problem, which carries a high morbidity and mortality [1]. CKD is characterized by a progressive loss of renal function, chronic inflammation, oxidative stress, vascular remodeling, and glomerular and tubulointerstitial scarring. CKD treatment still represents a clinical challenge. Diabetic nephropathy (DN) is the leading cause of CKD and end-stage renal disease (ESRD) [2]. The renin-angiotensin-aldosterone system (RAAS) is a major pathway involved in the pathogenesis and progression of DN [3, 4], and RAAS blockade is an effective therapeutic strategy to reduce proteinuria and slow progression of diabetic and non-diabetic CKD. However targeting the system sets off compensatory mechanisms that may increase angiotensin II, aldosterone, or renin, and partial RAAS blockade does not prevent progression in all CKD patients. Angiotensin II (AT II) is the key mediator of the RAAS [5–7]. Animal models of experimental diabetes, clinical trials, and metaanalysis have clearly demonstrated the effectiveness of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) therapy to improve glomerular/tubulointerstitial damage, reduce proteinuria, and decrease CKD progression, independently of blood pressure (BP) control [8–13]. Dual RAAS blockade with ACEI plus ARB inhibits compensatory AT II activity resulting from ACE-independent pathways and limits compensatory AT production induced by AT1 receptor blockade. This combination reduced proteinuria by 25–45% in DN [14–16]. Results are worse for DN with diminished kidney function or nonproteinuric CKD with ischemic renal injury, probably due to advanced structural renal changes [13, 17, 18] and adverse effects; such acute deterioration of renal function or hyperkalemia is more frequent. The aldosterone antagonists spironolactone and eplerenone reduce albuminuria by 30–60% and slow CKD progression in experimental models [19–21] and clinical studies [22–25] in DN. These agents abrogated the “aldosterone breakthrough” phenomenon and its proinflammatory and profibrotic effects. ACEI/ARB therapy increases renin. Aliskiren, a direct renin inhibitor, was beneficial in animal models of diabetic/hypertensive nephropathy [26, 27] and
reduced albuminuria in clinical DN [28]. In a multicenter and double-blind, randomized clinical trial in hypertensive type 2 DM patients with nephropathy, aliskiren plus losartan at maximal dose was 20% more effective than losartan/placebo to reduce albuminuria without adverse effects, independent of BP control [29].

A number of other strategies have been tried. Adequate BP and glucose control are part of standard care of DN patients. Intensive glucose control has more impact on GFR if early instituted in patients with type 1 DM but this may not necessarily apply to patients with type 2 DM or with advanced CKD [30]. A trial of the vitamin D activator paricalcitol missed the primary endpoint of albuminuria reduction in DN and caused a transient decrease in eGFR [31]. The nephroprotective effect of statins on CKD found in experimental models has not been conclusively proven in clinical studies [32]. A 1-year dose-ranging study of pirfenidone suggested better preservation of eGFR by pirfenidone in a small number of diabetic nephropathy patients [33]. The selective endothelin antagonist atrasentan reduced fenidone in a small number of diabetic nephropathy patients [34]. The nephroprotective effect of statins on CKD found in experimental models has not been conclusively proven in clinical studies [32]. A 1-year dose-ranging study of pirfenidone suggested better preservation of eGFR by pirfenidone in a small number of diabetic nephropathy patients [33]. The selective endothelin antagonist atrasentan reduced albuminuria in a short-term (8 weeks) study in a small number of diabetic patients while receiving RAS inhibitors but did not assess long-term renal function [34]. Heart failure patients or with peripheral edema were excluded.

In spite of all this experimental and clinical evidence, there are 35–40% of patients with DN that progress to renal failure or with peripheral edema were excluded.

ROS production in response to hyperglycemia, protein kinase C (PKC), advanced glycosylation end products (AGEs), free fatty acids, inflammatory cytokines, and TGF-beta1 contributes to these changes [43, 44, 52–54]. These stimuli activate the NADPH/NADPH oxidase system in renal cells. Oxidative stress induced by hyperglycemia or glucose degradation products may cause leukocyte or renal cell apoptosis and release of extracellular matrix [52, 55–59]. PKC activates NF-kappaB, extending the inflammatory response [60]. TGF-beta signaling is key to the excessive matrix formation [61, 62]. The activation of Nrf2 is increased in diabetic nephropathy and can ameliorate mesangial damage via partial inhibition of TGF-beta1 and reduction of extracellular matrix deposition [63]. ACE inhibitors lower TGF-beta in urine from DN patients. In rat DN glomerular HO-1 is increased, evidencing oxidative stress [52, 64]. ROS can activate several transcription factors such as NF-kappaB, AP-1, Sp1, which in turn affect the expression of mediators of inflammation, fibrosis, and cell death [52, 65] (Figure 1(a)).

ROS also contribute to renal injury in experimental glomerulonephritis. In experimental anti-Thy 1 glomerulonephritis, ROS enhance cell proliferation and matrix accumulation and fibrosis and this is improved by the antioxidant alpha-lipoic acid [66, 67]. ROS also regulate the immune response [68]. In nephrotoxic nephritis, neutrophils promote glomerular TNF-alpha expression via H2O2 production [69]. TNF-alpha is a key mediator of glomerular injury [70]. Interstitial inflammatory leukocytes in proliferative glomerulonephritis locally generate ROS and contribute to sodium retention [71]. Angiotensin II promotes ROS-mediated F-actin cytoskeleton rearrangement, resulting in podocyte injury [72]. In cultured podocytes AT1R signaling activates Rac-1 and NADPH oxidase to produce additional ROS and downregulates the antioxidant protein peroxiredoxin (Prdx2) [73]. In experimental passive Heymann nephritis, a model of membranous nephropathy, C5b-9 activation promotes ROS-mediated injury in glomerular cells [74, 75]. In this regard, evidence for oxidative stress, the glomerular neoexpression of aldose reductase (AR) and SOD2, and the appearance of anti-AR and anti-SOD2 autoantibodies was recently reported in human membranous nephropathy suggesting that oxidative stress may generate new autoimmune targets [76].

In lupus nephritis, multiple abnormalities in T and B cells lead to autoimmune renal inflammation and ROS production [77]. Nrf2-knockout mice showed impaired antioxidant activity, increased oxidative stress, and a lupus-like autoimmune nephritis with glomerular injury, impaired kidney function, and a shortened lifespan. Thus, Nrf2 deficiency could lead to systemic autoimmune inflammation with enhanced lymphoproliferation [78]. In antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, ANCA-activated neutrophils and monocytes release MPO and generate ROS, producing endothelial and tissue damage [79, 80]. ANCA also promote ROS-dependent dysregulation of neutrophil apoptosis [81] (Figure 1(a)).

In proteinuric nephropathies and independently of etiology, the presence of albumin in urine activates proximal

### 2. Oxidative Stress and Kidney Disease

Oxidative stress and inflammation promote kidney and vascular injury [37–42]. Several factors induce ROS in renal cells, such as inflammatory cytokines, Toll-like receptors, Angiotensin II, bradykinin, arachidonic acid, thrombin, growth factors, and mechanical pressure. NADPH oxidases, now renamed Nox enzymes, are key ROS generators in response to these stimuli [43, 44].

In acute kidney injury (AKI) induced by ischemic reperfusion injury, sepsis or acute rejections ROS contribute to endothelial and tubular injury [45, 46]. In murine models of AKI, bardoxolone methyl decreased functional and structural renal injury and increased the expression of protective genes (Nrf2, PPARy, HO-1) on glomerular endothelium, cortical peritubular capillaries, tubules, and interstitial leukocytes [47].

ROS contribute to hypertension-induced kidney and vascular injury [41, 43, 48, 49]. The chronic complications of diabetes are characterized by a defect in Nrf2 signaling and its adaptive response to oxidative stress. This is a potential mechanism for cellular stress hypersensitivity and tissue damage [50]. Diabetic nephropathy is characterized by initial hyperfiltration, albuminuria and subsequent loss of renal function, thickening of basement membranes, expansion of mesangial matrix and interstitial fibrosis, and podocytes

and renal cell damage [51]. ROS production in response to hyperglycemia, protein kinase C (PKC), advanced glycosylation end products (AGEs), free fatty acids, inflammatory cytokines, and TGF-beta1 contributes to these changes [43, 44, 52–54]. These stimuli activate the NADPH/NADPH oxidase system in renal cells. Oxidative stress induced by hyperglycemia or glucose degradation products may cause leukocyte or renal cell apoptosis and release of extracellular matrix [52, 55–59]. PKC activates NF-kappaB, extending the inflammatory response [60]. TGF-beta signaling is key to the excessive matrix formation [61, 62]. The activation of Nrf2 is increased in diabetic nephropathy and can ameliorate mesangial damage via partial inhibition of TGF-beta1 and reduction of extracellular matrix deposition [63]. ACE inhibitors lower TGF-beta in urine from DN patients. In rat DN glomerular HO-1 is increased, evidencing oxidative stress [52, 64]. ROS can activate several transcription factors such as NF-kappaB, AP-1, Sp1, which in turn affect the expression of mediators of inflammation, fibrosis, and cell death [52, 65] (Figure 1(a)).

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In proteinuric nephropathies and independently of etiology, the presence of albumin in urine activates proximal
AGE-RAGE
PKC
RAAS activation
Increased vasoconstrictors
NADPH oxidase
Vasoconstriction
Ischemia
Salt retention
Salt-sensitive hypertension
Podocyte, mesangial, endothelial, and tubular cell damage
Tubular atrophy
Glomerulosclerosis
Tubulointerstitial fibrosis
Tubulointerstitial inflammation
Albuminuria
Loss of kidney function
Progression to ESRD
Chronic immunological disorder
Complement system activation
Antibody production (auto-anti-Abs, ANCA)
Growth factors
Cytokines
Inflammatory cells
JAK/STAT signaling
↑ ROS
↑ TGF-β1
↑ ECM/EMT
Cytokines
Figure 1: Continued.
Figure 1: Overview of interrelation of ROS with other key pathogenic factors in kidney disease. (a) Role of ROS in diabetic nephropathy and immune-mediated glomerulonephritis. ROS are induced in renal cells in response to high glucose, AGE, and cytokines. PKC, NADPH oxidase, and mitochondrial metabolism are key to ROS generation. ROS activate signal transduction cascade and transcription factors, leading to upregulation of genes and proteins involved in renal cell injury, glomerular and interstitial extracellular matrix deposition, and recruitment of inflammatory cells, promoting albuminuria and progression of chronic kidney disease. (b) Role of albuminuria and ROS in tubular damage and progression of CKD. Albuminuria injures PTC and activates them to release chemokines that attract macrophages and promote tubulointerstitial fibrosis. Membrane NADPH oxidase is the main source of the ROS. It is possible the generation of other reactive species, as carbonyl groups derived from abnormal oxidation of albumin and fatty acids bound to albumin. Abs: antibodies, AGE: advanced glycation end products, ANCA: antineutrophil cytoplasmic antibodies, ECM: extracellular matrix, EMT: epithelial-mesenchymal transition, ESRD: end-stage renal disease, MCP-1: monocyte chemoattractant protein-1, NADPH: nicotinamide adenine dinucleotide phosphate, NF-kappa B: nuclear factor kappa B, PKC: protein kinase C, PTC: proximal tubular cell, RAAS: renin-angiotensin-aldosterone system, ROS: reactive oxygen species, RCG: reactive carbonyl groups, TGF-β1: transforming growth factor beta 1.
Humans.

Bardoxolone methyl, also known as CDDOMe, is a triterpenoid that activate the ARE-Keap1-Nrf2 pathway [91]. Bardoxolone, named triterpenoids, are potent anti-inflammatory agents [91–93]. Synthetic analogues of oleanolic acid, of proliferation, promotes differentiation and apoptosis induction [91–93]. Synthetic analogues of oleanolic acid, named triterpenoids, are potent anti-inflammatory agents that activate the ARE-Keap1-Nrf2 pathway [91]. Bardoxolone methyl, also known as CDDOMe, is a triterpenoid whose nephroprotective action has been recently explored in humans.

3. Oxidative Stress and the Keap1-Nrf2 Pathway

Reactive oxygen species (ROS) include superoxide anion (SOA), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical. ROS are formed continuously as by-product of aerobic metabolism. Sources of ROS include the mitochondrial electron transport chain, metabolism of arachidonate by cyclooxygenases or lipooxygenases, cytochrome P450 enzymes, NADPH oxidases, or nitric oxide synthetases [88]. ROS contribute to killing bacteria, and genetic defects of NADPH oxidase cause chronic granulomatosis [89]. However, ROS may cause chemical damage to DNA, proteins, and unsaturated lipids and lead to cell death. ROS contribute to multiple pathologic processes [48, 90]. In this regard, homeostasis is maintained through a complex set of antioxidants mechanisms that prevent oxidative stress-induced injury. The main mechanisms are enzymes that catalyze antioxidant reactions: glutathione peroxidase, superoxide dismutase, catalase, Hem-oxygenase (HO-1), NADPH-quinone oxidoreductase and glutamate-cysteine ligase. These enzymes are encoded by stress-response genes or phase 2 genes that contain antioxidant response elements (AREs) in their regulatory regions [88, 91]. Nrf2 is the principal transcription factor that binds to the ARE promoting transcription. Actin-tethered Keap1 is a cytosolic repressor that binds to and retains Nrf2 in the cytoplasm, promoting its proteasomal degradation. Inducers of phase 2 genes modify specific cysteine residues of Keap1 resulting in conformational changes that render Keap1 unable to repress Nrf2. Consequently, Nrf2 activates the transcription of phase 2 genes. Oleoanolic acid activates the ARE-Keap1-Nrf2 pathway, resulting in reduced proinflammatory activity of the IKK-beta/NF-kappaB pathway, increases production of antioxidant/reductive molecules, and decreases oxidative stress, thereby restoring redox homeostasis in areas of inflammation. In various cell lines, this results in inhibition of proliferation, promotion of differentiation and apoptosis induction [91–93].

4. Antioxidant Agents in Kidney Disease

Epidemiological studies have demonstrated association between inflammatory and oxidative stress markers with cardiovascular and renal outcomes in CKD and ESRD [40, 94–96]. Experimental data in animal models of renal disease suggest beneficial effects of antioxidants agents, but results in human studies are limited and controversial.

In early experimental diabetes mellitus in hypertensive rats, the administration of tempol, an antioxidant SOD mimic, corrected the oxidative imbalance and improved oxidative stress-induced renal injury, decreasing albuminuria and fibrosis [97]. Similar protection was afforded by the antioxidant N-acetyl-L-cysteine (thiol) and kallistatin in Dahl salt-sensitive rats [98, 99]. In spontaneously hypertensive rats, a lifelong antioxidant-rich diet diminished the severity of hypertension, improved oxidative stress and ameliorated abnormalities of antioxidant enzyme expressions and activities in contrast to regular diet [100]. In summary, in models of hypertensive rats, synthetic and natural antioxidants induced renal and endothelial protection with reduction of oxidative stress. In a model of ischaemia reperfusion and cyclosporin toxicity after unilateral nephrectomy, the blockage of the mitochondrial enzymes monoamine oxidases with pargyline 28 days following surgery prevented H$_2$O$_2$ production and improved renal function and renal inflammation (lower IL-1β and TNF-α gene expression) [101]. Pargyline administrated before ischemia reperfusion significantly reduced apoptosis, necrosis, and fibrosis. This effect was associated to decreased expression of TGF-β1, collagen types I, III, and IV and to the normalization of SOD1, catalase, and inflammatory gene expression. In models of renal chronic failure (5/6 nephrectomy rats) [102], AST-120, an oral carbonic adsorbent, improved the oxidative stress in endothelial cells, measured as oxidized/unoxidized albumin ratio. This effect was reached reducing the blood levels of indoxyl sulfate, a uremic toxin that induces ROS. In another model of remnant kidney, the administration of omega-3 fatty acids, an effective compound in mitigating atherosclerosis, significantly lowered several components of oxidative stress and markers of inflammatory and fibrotic response. Furthermore, it attenuated tubulointerstitial fibrosis and inflammation in the remnant kidney [103]. In anti-Thy1 glomerulonephritis, the treatment with parthenolide, an anti-inflammatory agent related to the triterpenoid family, diminished renal inflammation via NF-kappaB inhibition, decreased MCP-1 and iNOS, and improved proteinuria, tubular, and glomerular damage [104]. The beneficial effect of exogenous antioxidants shown in animal models with hypertension or chronic renal failure has not been demonstrated in people with clinical hypertension [96, 105] or CKD.

5. Nephroprotection by Bardoxolone Methyl

Bardoxolone was initially described as an agent that protected cells from radiation-induced damage (radiation mitigator) through Nrf2-dependent and -independent pathways [106]. In humans, its potential antineoplastic activity was...
BARD promotes activation of the Nrf2 transcription factor, that is released of the inhibitory Keap1 protein and migrates to the nucleus where it regulates transcription of genes containing ARE sequences in their promoters. These phase 2 response genes are collectively involved in the reduction of ROS and inhibition of NF-kappaB. Thus, BARD could promote renal protection through antioxidants and anti-inflammatory effects be promoting the activity of the Nrf2 transcription factor and inhibiting the activity of the NF-kappaB transcription factor. ACE/ACEIs: angiotensin converting enzyme/angiotensin converting enzyme inhibitors, ARBs: angiotensin receptor blockers, AREs: antioxidant response elements, BARD: bardoxolone methyl, CKD: chronic kidney disease, DRI: direct renin inhibitor, mineralocorticoid receptor antagonists, Keap1: Kelch-like ECH-associated protein 1, MRA: mineralocorticoid receptor antagonists, NF-kappaB: nuclear factor kappa B, Nrf2: nuclear factor (erythroid derive 2)-like 2, RAAS: renin-angiotensin-aldosterone system, ROS: reactive oxygen species, TGFβ-1: transforming growth factor beta 1.

evaluated. In phase 1 trials in oncologic patients, bardoxolone unexpectedly improved kidney function, assessed as serum creatinine and creatinine clearance, especially in patients with previous CKD. These findings lead to evaluate potential nephroprotective actions in patients with CKD and type 2 DM, first in an exploratory phase II open-label trial and then in a larger randomized clinical trial. In the first trial [107], 20 patients older than 18 years, with moderate-severe diabetic CKD, were evaluated after 8 weeks of bardoxolone at increasing oral doses of 25 to 75 mg/day. Notably, there
was a significant increase in estimated GFR at 4 weeks (+2.8 mL/min/1.73 m²) with 25 mg/day and at 8 weeks (+7.2 mL/min/1.73 m²) with 75 mg/day. Serum creatinine and BUN decreased and creatinine clearance increased, without changes in total excretion or tubular secretion of creatinine. Unfortunately, GFR was not measured. Blood pressure did not change, and albuminuria had a small albeit not statistically significant increase. Markers of vascular injury and inflammation were improved by treatment with bardoxolone, suggesting a potential beneficial effect on endothelial injury. There were not changes in urine NGAL or NAG adjusted for creatinine concentration, suggesting lack of significant renal toxicity associated to bardoxolone. There were few adverse effects, mainly muscle spasms and a self-limited increase of hepatic enzymes without a true hepatic toxicity. A short followup and an open-label design without control group are major limitations of this study, and they do not allow drawing solid conclusions about the efficacy and long-term safety of this drug on relevant renal outcomes.

The beneficial effect on eGFR was confirmed in a larger, multicenter, double-blind, randomized trial [108]. This trial randomized 227 patients with moderate-severe CKD and type 2 DM, with stable treatment with ACEI/ARB, to bardoxolone 25, 75 or 150 mg/day or placebo for 52 weeks. Patients were categorized by GFR, urinary albumin-creatinine ratio (UACR), and HbA1c. Patients with hepatic dysfunction or recent cardiovascular events were excluded. A significant improvement in the primary endpoint (change of GFR at 24 weeks) was observed in all bardoxolone groups (+8.2, +11.4 and +10.4 mL/min/1.73 m² resp.) versus 0 mL/min/1.73m² in the placebo group. The secondary endpoint (change of GFR at 52 weeks) also was significantly improved in bardoxolone groups (+5.8, +10.5 and +9.3 mL/min/1.73 m² resp. versus 0). More patients in the placebo group had a GFR decrease ≥25% with respect to baseline value at 24 and 52 weeks. Additionally, serum BUN, phosphorus, and uric acid were significantly lower at 24 and 52 weeks in all bardoxolone groups when compared to placebo.

Potential unwanted effects included a mild but significant increase of UACR and decreased serum magnesium. UACR increased in patients receiving 75 or 150 mg/day bardoxolone versus placebo. This was observed at 24 and 52 weeks of treatment, but UACR decreased when patients stopped the therapy, suggesting that this effect is reversible. There was an inverse correlation between changes in serum BUN, phosphorus, uric acid, magnesium, and changes in eGFR, as well as a direct correlation between changes in eGFR and changes in UACR, suggesting that changes in eGFR may be the basis for the other observed changes. Interestingly, there was a trend toward higher systolic BP values in the 75 mg bardoxolone group, which was observed despite weight loss and that will merit close attention in further trials. The main adverse effects were muscle spasms (63% of patients in the 75 mg group) and nausea (25%).

Another significant effect was loss of body weight. This appears to be related to decreased appetite and/or nausea and may be a welcome addition to the therapeutic armamentarium for patients with increased body mass index (BMI). Indeed, weight loss was more evident in patients with higher (>35 kg/m²) BMI (mean change −10 kg). However, and perhaps worryingly, it was also observed in patients with normal BMI (−3 kg over 52 weeks).

The increased eGFR and effects on systolic BP and albuminuria are interesting results on surrogates renal variables which requires more long-term studies. These parameters and, more importantly, cardiac and renal hard end-points (cardiovascular death and progression to ESRD) will be studied at 2 years of followup in an ongoing randomized clinical trial in 1600 patients older than 18 years with advanced CKD (stage 4) and type 2 DM [109]. This study will compare bardoxolone versus placebo in patients receiving standard of care.

6. Conclusions and Recommendations

RAAS blockade is the mainstay of current therapy to slow progression of diabetic and nondiabetic CKD, but this strategy is frequently not enough. Consequently, an important number of patients progress to ESRD. There is solid experimental evidence for a key role of ROS and oxidative stress and their interplay with RAAS and inflammation, in the pathogenesis of CKD. Bardoxolone methyl, a novel synthetic triterpenoid with antioxidant and anti-inflammatory properties, has shown to improve kidney function in patients with advanced DN already receiving RAAS blockers, with few adverse events. This may be a welcomed addition to the therapeutic armamentarium if data are confirmed in larger, longer trials (Figure 2). However, the relative importance and eventual management of the observed influence of bardoxolone on UACR, magnesium, and body weight must be further studied.

Acknowledgments

This paper is supported by the following Grants: ISCIII and FEDER funds CP04/00060, FIS PS09/00447, Sociedad Española de Nefrología, ISCIII-RETIC REDinREN/RD06/0016, Comunidad de Madrid/FRACM/S-BIO0283/2006, S2010/BMD-2378, Programa Intensificación Actividad Investigadora (ISCIII/Agenica Lain-Entralgo/CM) to AO, and ISCIII-Redes RECAVA (RD06/0014/0035), ISCIII funds PI10/00072, EUS2008/03565 to JE and Fundacion Lilly, cvREMOD.

References


Review Article

Induction of Oxidative Stress in Kidney

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Received 1 December 2011; Revised 27 January 2012; Accepted 6 February 2012

Academic Editor: Ayse Balat

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Oxidative stress has a critical role in the pathophysiology of several kidney diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress-related mediators, and inflammation. Several systemic diseases such as hypertension, diabetes mellitus, and hypercholesterolemia; infection; antibiotics, chemotherapeutics, and radiocontrast agents; and environmental toxins, occupational chemicals, radiation, smoking, as well as alcohol consumption induce oxidative stress in kidney. We searched the literature using PubMed, MEDLINE, and Google scholar with “oxidative stress, reactive oxygen species, oxygen free radicals, kidney, renal injury, nephropathy, nephrotoxicity, and induction”. The literature search included only articles written in English language. Letters or case reports were excluded. Scientific relevance, for clinical studies target populations, and study design, for basic science studies full coverage of main topics, are eligibility criteria for articles used in this paper.

1. Introduction

Free radicals are chemical species with a single unpaired electron, which is highly reactive as it seeks to pair with a new free electron, and as a result of these reactions, other free radicals or paired electrons occur and radical feature may be lost. If newly formed free radical occurs, it is also unstable and it can react with another molecule to produce another free radical or a nonradical molecule occurs because of the paired electrons of the newly formed molecule. Thus, a chain reaction of free radicals occurs, leading to damaging biological systems and tissues. In aerobic conditions, all biological systems are exposed to oxidative stress (OS), either generated internally or as by-products. The great majority of these free radicals are mainly oxygen radicals and other reactive oxygen species (ROS) [1].

The well-known ROS are superoxide ion (O$_2^*$), hydrogen peroxide (H$_2$O$_2$), and peroxyl radical (OH•), and the reactive nitrogen species (RNS) are nitric oxide (NO) and peroxynitrite (ONOO•). Peroxynitrite generates from rapid chemical interaction between NO and O$_2^*$. Main sites of ROS produced in living organisms are mitochondrial electron transport system, peroxisomal fatty acid, cytochrome P-450, and phagocytic cells [2–6]. Several extracellular and intracellular factors such as hormones, growth factors, proinflammatory cytokines, physical environmental factors (like ultraviolet irradiation), nutrient metabolism, and the detoxification of various xenobiotics affect production of OS [7–13].

In physiological conditions, ROS produced in the course of normal conditions are completely inactivated by cellular and extracellular defence mechanisms. This means that normally there is a balance between prooxidant (or oxidant) and antioxidant defence systems. In certain pathological conditions, increased generation of ROS and/or depletion of antioxidant defence system leads to enhanced ROS activity and OS, resulting tissue damage. OS causes tissue damage by different mechanisms including promoting lipid peroxidation, DNA damage, and protein modification. These processes have been implicated in the pathogenesis of several systemic diseases including kidney.

Several systemic diseases such as hypertension, diabetes mellitus, metabolic syndrome, and hypercholesterolemia; infection; antibiotics and chemotherapeutic agents, and radiocontrast agents mainly excreted from kidney; and environmental toxins especially heavy metals such as lead and mercury, occupational chemicals such as urban fine particles, radiation, smoking, as well as alcohol consumption induce renal OS. The kidney is an organ highly vulnerable to
damage caused by ROS, likely due to the abundance of long-chain polyunsaturated fatty acids in the composition of renal lipids. In recent years, OSs have become one of the most popular topics in research of molecular mechanism of renal diseases. The aim of this paper is to summarize the conditions inducing OS in kidney and molecular mechanisms of this induction and kidney damage.

2. Diabetes Mellitus and Induction of Oxidative Stress in Kidney

Recent years, diabetes and diabetic kidney disease continue to increase worldwide. In the USA, diabetes-associated kidney disease is a major cause of all new cases of end-stage kidney disease. All diabetic patients are considered to be at risk for nephropathy. Today we have not specific markers to expect development of end-stage renal disease. Clinically control of blood sugar level and blood pressure regulations are important two parameters to the prevention of diabetic nephropathy [14, 15].

There are huge amount of in vitro and in vivo studies regarding explanation of mechanism of diabetes-mellitus-induced nephropathy. All of these mechanisms are a consequence of uncontrolled elevation of blood glucose level. Currently the proposed mechanism is the glomerular hyperfiltration/hypertension hypothesis. According to this hypothesis, diabetes leads to increased glomerular hyperfiltration and a resultant increased glomerular pressure. This increased glomerular pressure leads to damage to glomerular cells and to development of foci and segmental glomerulosclerosis [16, 17]. Angiotensin II inhibitors reduce glomerular pressure and prevent albuminuria. Increased angiotensin II level induces OS through activation of NADPH oxidase, stimulating inflammatory cytokines, and so forth . . . [18, 19].

The mechanism by which hyperglycemia causes free radical generation thus causes OS to be complex. Increased blood glucose promotes glycosylation of circulator and cellular protein and may initiate a series of autooxidation reactions that culminate in the formation and accumulation of advanced glycosylation end-products (AGEs) in tissues. The AGEs have oxidizing potential and promote tissue damage by oxygen-free radicals [20].

In experimental studies, formation of OS increases because of high level of blood glucose. Sadi et al. showed that in diabetic rat kidney antioxidant enzyme, namely, catalase (CAT) and glutathion peroxidase (GSHPx), activities were found to be reduced; however, α-lipoic acid and vitamin C administration increased these antioxidant enzyme activities [21]. Increased OS is the common finding in tissues effecting from diabetes, including kidney. Reddi et al. showed that transforming growth factor β1 (TGF-β1) is prooxidant and Se (selenium) deficiency increases OS via this growth factor. In addition Se deficiency may simulate hyperglycemic conditions. Se supplementation to diabetic rats prevents for formation of OS and renal structural injury [22]. Chen et al. showed that nitrosative stress increases in diabetic rat model [23]. These results show the induction of oxidative and nitrosative stress in rat kidney. These may have a role in pathophysiology of diabetes-induced morphological and functional changes of kidney.

3. Hypertension, Hypercholesterolemia, Obesity, and Aging Induce Oxidative Stress in Kidney

Hypertension is one of the major causes of development of renal failure. Key regulator of this pathology is OS. Renal artery stenosis is the most common cause of secondary hypertension and may lead to deterioration of renal function and ischemic nephropathy. Chade et al. showed that a cross-talk between hypoperfusion and atherosclerosis to interactively increased OS, inflammation, and tubular injury in the stenotic kidney [24]. In experimental atherosclerotic renovascular disease (simulated by concurrent hypercholesterolemia and renal artery stenosis), it was reported that the activity of both CuZn and MnSOD isoforms was significantly decreased; however, protein expression of both the NAD (P)H-oxidase subunits p67phox and p47phox, nitrotyrosine, inducible nitric oxide synthase (iNOS), and nuclear factor kappa-B (NFκB) increased. Furthermore, tubular and glomerular protein expression of nitrositroserine is significantly elevated [25]. Chronic blockade of OS with antioxidant improves OS in kidney. All of these molecular abnormalities suggest increased OS in rat kidney.

Noeman et al. showed that high-fat diet-induced obesity is accompanied by increased hepatic, cardiac, and renal tissue OS, which is characterized by reduction in the antioxidant enzymes activities and glutathione levels, that correlate with the increase in MDA and protein carbonyl (PCO) levels [26]. Increased cytokine release (inflammation-related cytokines such as tumor necrosis factor-α and adiponectin) and renal macrophage infiltration have been shown to contribute to renal injury in models of obesity. Chow et al. reported that monocyte chemoattractant protein-1 (MCP-1) is a potent stimulator of macrophage recruitment. It is increased in adipose tissue during obesity and in diabetic kidneys, suggesting that inflammation of these tissues may be MCP-1 dependent [27]. Knight et al. also showed an increase in renal macrophage-specific CD68-positive staining in a model of obesity and hypertension [28]. From these results, we can say that macrophages are the source of increased OS and renal injury in diabetes and obesity-induced renal injury. Aging is associated with increased OS. Most of age-dependent changes in the kidney such as excessive fibrosis, a general lack of regenerative ability, and an increase in apoptosis in cells that determine healthy renal functions are often related to excess OS [29]. At a molecular level, with aging increased mutations in nuclear and mitochondrial DNA (mtDNA), increased lipofuscin and AGES, increased OS, and increased apoptosis have been observed. Proximal tubular cells contain large numbers of mitochondria and are the most reliant upon oxidative phosphorylation and most susceptible to oxidant-induced apoptosis and mutations [30]. Recent studies showed that antiaging gen, klotho, is important in renal aging and OS-induced renal damage. The klotho gene encodes the klotho protein, a single transmembrane protein
of the beta-glycosidase family [31]. Mice overexpressing klotho exhibit an extension in lifespan and resistance to oxidative injury. Klotho is predominantly expressed in the kidney, with its highest expression in cells of the distal convoluted tubule [32, 33]. Overexpression of klotho has been found to enhance resistance to OS through the upregulation of manganese superoxide dismutase (MnSOD). Yamamoto et al. found that klotho modulates MnSOD in a FoxO-dependent process [34]. MnSOD is found within the mitochondria where it acts as a primary scavenger of oxidants.


Most clinical and experimental studies have shown that OS is increased in kidney and systemic circulation. Huang et al. reported that the activities of catalase and manganese superoxide dismutase were elevated in early stage of ethylene glycol-induced urolithiasis model in rats; however, on day 42 almost all antioxidant enzyme activities were attenuated except those of CAT. In this experiment, the possible mechanism that causes free radical elevation in the kidney may be different in the course of ethylene glycol-induced urolithiasis. Initially systemic circulation may bring the toxic substances to the kidney, and eventually these substances cause to produce free radicals. In the late stage progressive accumulation of leukocytes and defective antioxidant enzyme activities may cause kidney to remain under huge amount of OS [35, 36]. In our experimental urolithiasis studies, we showed decreased antioxidant enzyme activities and involvement of NFκB and p38-MAPK (mitogen-activated protein kinase) signaling pathways, related to OS in rat kidney [37–40]. In vitro cell culture studies using proximal tubular origin line derived from pig proximal tubules (LLC-PK1), and collecting duct origin Madin-Darby canine kidney (MDCK) cell lines, it has been reported that calcium phosphate crystals cause cellular injury by increasing ROS [41].

Today, extra corporeal shock wave lithotripsy (ESWL) is widely used for the treatment of renal stones in selected renal cases. In our work, we showed increased expression of inducible NO synthase (iNOS) and NFκB, indirect evidence of increased OS [42]. Recently, Gecit et al. showed that ESWL treatment produces OS and causes impairment in the antioxidant and trace element levels in the kidneys of rats [43]. ESWL is associated with greater prevalence of hypertension [44]. Ischemia insult and increased renal OS and consequent endothelial dysfunction may be possible mechanism of hypertension after ESWL.

Urinary obstruction, especially ureteral obstruction due to urolithiasis, is a common urological problem seen in urology practice. Unilateral ureteral obstruction (UUO) leads to decreased renal MnSOD and CAT protein expression in a time-dependent manner. Increased 4-hydroxynonenal (4-HNE) stain for ROS products in the renal tubulointerstitial compartment occurred after 4 hr of UUO in the kidney. The authors explain renal tubular apoptosis in UUO rat model explained by increasing ROS in this study [45]. Various markers of OS increase in UUO rat kidneys such as the oxidatively damaged protein product Ne-carboxymethyl-lysine (CML); the marker of DNA oxidant damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG); and lipid peroxidation markers such as malondialdehyde (MDA), 8-iso prostaglandin F2α (8-iso PGF2α), and 4-HNE or 4-hydroxy-hexenal (4-HHE). OS response molecules like heat shock protein-70 (HSP-70), heat-shock protein-27, and heme oxygenase-1 (HO-1) [46–51] are strongly expressed after UUO. Mice that are genetically deficient endogenous antioxidant enzyme CAT are more susceptible to UUO-induced renal damage than normal wild type mice. Furthermore, increased renal concentrations of ROS have been observed in obstructed kidneys, together with decreased activities of the major protective antioxidant enzymes SOD, CAT, and glutathione peroxidase [52, 53]. UUO-induced nephrotoxicity and renal fibrosis is thought to be secondary to increased OS in kidney. In the literature there is some information about the amelioration of antioxidant and reactive oxygen scavenger agents against UUO-induced renal damage [54, 55]. For an excellent detailed review, please refer to [56].

Infection is another inducer of OS in kidney. There are a lot of experiments showing the increased oxidative stress and decreased antioxidant defense mechanism, antioxidant enzyme systems in kidney due to infection [57–60]. ROS are important mediators exerting toxic effects on various organs, including kidney during ischemia-reperfusion (IR) injury. A large body of evidences indicate the role of increased OS in the kidney and protective role of antioxidants and ROS scavengers in IR injury-induced nephropathy in the literature [61–63]. OS also has a role as a mediator of injury in chronic allograft tubular atrophy and interstitial fibrosis in rat kidney [64]. Renal transplantation is another OS inducer in kidney in human and animals. OS increases in transplanted kidney because of pretransplant and posttransplant conditions. If there is preexisting diseases such as chronic kidney failure, inflammation, and diabetes mellitus, kidneys are more sensitive to OS during reperfusion injury. Postoperative immunosuppressive agents are among many risk factors inducing OS in kidney [65].

5. Antibiotics, Antineoplastic Agents, Immunosuppressants, Analgesics, Nonsteroidal Antiinflammatory Drugs, and Radiocontrast Agents Induce Oxidative Stress in Kidney

Antibiotics, commonly used aminoglycosides, are nephrotoxic agents. Their nephrotoxicity is mainly attributed to induction of OS and depletion of antioxidant enzyme activities in kidney. In our experiments, we showed that iNOS/NFκB/p38MAPK pathway, OS taking place in this axis, is involved in gentamicin-induced nephrotoxicity [66, 67]. Our and other studies showed the protective effect of anti oxidants and reactive oxygen scavenger agents against gentamicin-induced nephrotoxicity [68–70].
Antineoplastic agents are commonly used for the treatment of metastatic cancers. Some of these are nephrotoxic. Excess ROS production and depressed antioxidant defence mechanism are responsible from nephrotoxicity. Cisplatin is the well-known and commonly used antineoplastic and nephrotoxic agent. Other nephrotoxic anticancer agents are carboplatin, methotrexate, doxorubicin, cyclosporine, and adriamycin. Immunosuppressant such as sirolimus and cyclosporine leads to nephrotoxicity via OS [71–80].

Cisplatin is one of the commonly used potent antitumor drugs and cisplatin-based combination protocols are used as front-line therapy for several human malignancies. Cisplatin is toxic to the renal proximal tubules and dose dependent [81, 82]. Several studies have reported the role of OS regarding cisplatin-induced nephrotoxicity. Cisplatin is known to accumulate in mitochondria of renal tubular epithelial cells together with ROS; renal tubular cell mitochondrial dysfunction is also important in cisplatin-induced nephrotoxicity. Mitochondria also continuously scavenge ROS via the action of antioxidant enzymes such as SOD, GSHPx, CAT, and glutathione S-transferase. Studies have demonstrated that cisplatin induces ROS in renal epithelial cells primarily by decreasing the activity of antioxidant enzymes and by depleting intracellular concentrations of GSH. In vitro studies using LLC-PK1 cells, which is characteristic of renal proximal tubular epithelium, also showed the role of ROS in cisplatin nephrotoxicity [83–89].

In this era, analgesics, especially paracetamol and acetaminophen (APAP), and nonsteroidal antiinflammatory drugs (NSAIDs) are widely used throughout the world. Paracetamol and APAP are nephrotoxic drugs. Several in vitro and in vivo studies showed that analgesics nephrotoxicity is caused by increased ROS in kidney. Zhao et al. showed the increased ROS, nitric oxide, and MDA levels, together with depleted glutathione (GSH) concentration in the kidney of rats. However, rhein, Chinese herb, can attenuate APAP-induced nephrotoxicity in a dose-dependent manner [90]. We showed in our experiment a significant increase in MDA and decreases in GSHPx, CAT, and SOD activities in APAP-treated rat kidneys. These findings support the induction of OS in rat kidney by APAP. Significant beneficial changes were noted in serum and tissue OS indicators in rats treated with strong antioxidant pinel hormone melatonin and curcumin [91, 92]. Ghosh et al. reported increased OS and TNF-alpha production in rat tissues [93]. Efrati et al. reported that diclofenac (NSAID) leads to nephrotoxicity by increasing intrarenal ROS in rat kidney, and antioxidant, N-acetylcysteine, prevents kidney damage [94]. Contrast-induced nephropathy (CIN) is a major clinical concern, particularly with imaging procedures. CIN is the third most common cause of acute kidney injury in hospitalized patients [95]. Experimental findings in vitro and in vivo illustrate enhanced hypoxia and the formation of ROS within the kidney following the administration of iodinated contrast media, which may play a role in the development of CIN. Studies indeed support this possibility, suggesting a protective effect of ROS scavenging or reduced ROS formation with the administration of N-acetyl cysteine and bicarbonate infusion, respectively [96–99].

6. Alcohol, Smoking, Environmental Toxins, Radiation, and Mobile Phones Induce Oxidative Stress in Kidney

Ethanol and its metabolites are excreted into urine, and its content in the urine is higher than that of the blood and the liver. Chronic alcohol administration decreases the renal tubular reabsorption and reduces renal function. Functional abnormalities of renal tubules may be associated with ethanol-induced changes in membrane composition and lipid peroxidation. Because of high content of long-chain-polyunsaturated fatty acids, kidney is highly sensitive to OS damage [100].

Recently it is reported that ethanol administration caused a significant decrease in the levels of antioxidant enzyme CAT, SOD, and GSHPx activities and increases MDA in kidney of the rats [101]. Shankar et al. showed that renal metabolism of ethanol via Cytochrome P450 2E1 (CYP2E1) and antiduretic hormone-1 led to production of renal OS, and activation of MAPK induces CYP24A1 resulting in reducing circulating 1,25 (OH)2 D3 concentrations [102]. Pathogenesis of aldosterone/salt-induced renal injury similarly is attributed to increased ROS and activation of MAPK in rat kidney [103]. In other studies, authors showed that chronic ethanol administration and cigarette smoke exposure may cause renal injury by increasing oxidative and nitrosative stress in rat kidney [104, 105].

Epidemiological studies have shown that smoking is an accelerating risk factor for the development of nephropathy, in which TGFβ1 plays a role in diabetic patients. Cell culture studies using mesangial cell showed that smoking could increase TGFβ1, probably due to increased oxidative stress and PKCβ (protein kinase C beta) activation. This finding supports the concept that smoking is a risk factor for development of diabetic nephropathy by increasing OS in kidney [106]. Similarly smoking and alcohol together increase OS and suppress antioxidant defence mechanism in kidney [104, 105].

In modern era, especially industrialised countries environmental toxins such as air pollutants, substances in stored foods, radiations, as well as heavy metals in waters especially in underdeveloped countries are major health problem. Ochratoxin A (OTA), a mycotoxin, produced by fungi of improperly stored food products, has been linked to the genesis of several disease states in both animals and humans. It has been reported as nephrotoxic, carcinogenic, teratogenic, immunotoxic, and hepatotoxic in laboratory and domestic animals [107–109]. In primary rat kidney cells and in vivo experiments, it has been shown that OTA induces OS and depletes antioxidant systems. These events might represent pivotal factors in the chain of cellular events leading into nephrotoxicity of OTA. Cadmium (Cd) is known to be a widespread environmental contaminant and a potential toxin that may adversely affect public health. Cigarette smoke and food (from contaminated soil and water) are important nonindustrial sources of exposure to Cd. Cd accumulates in the kidney because of its preferential uptake by receptor-mediated endocytosis of freely filtered and metallothionein
bound Cd (Cd-MT) in the proximal tubule. Internalised Cd-MT is degraded in endosomes and lysosomes, releasing free Cd into the cytosol, where it can generate ROS and activate cell death pathways [110].

Another environmental nephrotoxic agent is diazinon (O,O-diethyl-O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate). It is an organophosphate insecticide and has been used worldwide in agriculture and domestic for several years. Shah et al. showed that diazinon exposure depletes antioxidant enzymes and induces OS in rat kidney [111].

Increased air pollution as a result of industry is another life threatening health problem. Boor et al. showed renal, vascular, and cardiac fibrosis in rats exposed to passive smoking and industrial dust fibre amosite. Authors explain these changes by increased OS in these tissues [112]. Nanosized fraction of particulate air pollutants have been reported to translocate from the airways into the bloodstream and act on different organs such as lungs, heart, liver, and kidneys. Nemmar et al. examined the distribution and the pathological changes of diesel exhaust particles (DEPs) on systolic blood pressure (SBP), systemic inflammation, oxidative stress, and morphological alterations in lungs, heart, liver, and kidneys in Wistar rats. They showed that DEPs cause inflammation especially in lungs and pulmonary tissue, and these pathological changes are attributed to increased OS and inflammatory cytokines in these tissues [113]. Lead and cadmium nephrotoxicity are also related to increased OS in kidney [114, 115].

Radiation is an important inducer of OS. For diagnostic and therapeutic purposes, radiation is commonly used. Chronic OS after total body irradiation is thought to be the cause of radiation nephropathy in rats. Authors looked for evidence of OS after total-body irradiation in a rat model; focusing on the period before that there is physiologically significant renal damage. No statistically significant increase in urinary 8-isoprostan (a marker of lipid peroxidation) or carbonylated proteins (a marker of protein oxidation) was found over the first 42 days after irradiation, while a small but statistically significant increase in urinary 8-hydroxydeoxyguanosine (a marker of DNA oxidation) was detected at 35–55 days. In renal tissues, they found no significant increase in either DNA or protein oxidation products over the first 89 days after irradiation. They suggest that if chronic OS is a part of the pathogenesis of radiation nephropathy, it does not leave widespread or easily detectable evidence behind. Emre et al. investigated the effect of extremely low-frequency electromagnetic field (ELF-EMF) with pulse trains exposure on lipid peroxidation and hence oxidative stress in the rat liver and kidney tissue. They found increases in the levels of oxidative stress indicators, and the flow cytometric data suggested a possible relationship between the exposure to magnetic field and the cell death; however, there were significantly lower necrotic cell percentages in experimental animals compared to either unexposed or sham control groups [116]. These results suggest the inductive effect of radiation on OS in kidney.

For the last two decades, a large number of studies have investigated the effects of mobile phone radiation on the human and animal. Cellular target and tissue damage are different. Male reproductive system is among the most affected system [117, 118]. Increased OS plays a central role in radio-frequency-electromagnetic-waves- (RF-EMW-) induced tissue damage. Devrim et al. examined the effect of RF-EMW on oxidant and antioxidant status in erythrocytes and kidney, heart, liver, and ovary tissues from rats and possible protective role of vitamin C. It was observed that MDA level, xanthine oxydase (XO), and GSH-Px activities significantly increased in the EMR group as compared with those of the control group. In the kidney tissues, it was found that MDA level and CAT activity significantly increased, whereas XO and adenosine deaminase (ADA) activities decreased in the cellular phone group as compared with those of the control group. However, in the heart tissues, it was observed that MDA level, ADA, and XO activities significantly decreased in the cellular phone group as compared with those of the control group. They concluded that RF-EMR at the frequency generated by a cell phone causes OS and peroxidation in the erythrocytes and kidney tissues from rats. In the erythrocytes, vitamin C seems to make partial protection against the OS [119]. Ozguner et al. reported similar results in rat experiments and they also reported that preventive effect of Caffeic acid phenethyl ester (CAPE), a flavonoid-like compound, is one of the major components of honeybee propolis and melatonin against RF-EMW-induced nephrotoxicity [120–122].

7. Conclusion

There is huge amount of literature concerning the link between the OS and renal diseases. Systemic diseases such as hypertension, diabetes mellitus, and hypercholesterolemia; infection; antibiotics, chemotherapeutics, and radiocontrast agents; and environmental toxins, occupational chemicals, radiation, smoking, as well as alcohol consumption induce renal OS. The kidney is a highly vulnerable organ to damage caused by ROS, due to the abundance of long-chain-polyunsaturated fatty acids. Antioxidant and reactive oxygen scavengers have been shown to be effective in animals for protecting kidney, but it is hard to translocate these results to humans. This may be due to short duration of animal studies, dose differences between animals and humans, and different pathophysiologic processes between animals and humans. For understanding these steps, drugs will be developed to alter the main process(es) responsible for increased OS. In this paper, the conditions inducing OS in kidney and molecular mechanisms of this induction and kidney damage have been summarized. I hope that this paper will aid in the understanding of this complex system and directing new research effort.

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Research Article

Inflammation and Oxidative Stress in Obesity-Related Glomerulopathy

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Received 2 September 2011; Accepted 6 February 2012

Academic Editor: Ayse Balat

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Obesity-related glomerulopathy is an increasing cause of end-stage renal disease. Obesity has been considered a state of chronic low-grade systemic inflammation and chronic oxidative stress. Augmented inflammation in adipose and kidney tissues promotes the progression of kidney damage in obesity. Adipose tissue, which is accumulated in obesity, is a key endocrine organ that produces multiple biologically active molecules, including leptin, adiponectin, resistin, that affect inflammation, and subsequent deregulation of cell function in renal glomeruli that leads to pathological changes. Oxidative stress is also associated with obesity-related renal diseases and may trigger the initiation or progression of renal damage in obesity. In this paper, we focus on inflammation and oxidative stress in the progression of obesity-related glomerulopathy and possible interventions to prevent kidney injury in obesity.

1. Introduction

Obesity has become a heavy public health problem in the United States, with a prevalence among adults increasing to 32% from 13% between the 1960s and 2004 [1]. Currently, 66% of adults and 16% of children and adolescents are overweight or obese [1]. Although obesity has long been recognized as an independent risk factor for cardiovascular diseases and diabetes mellitus, newer research points to obesity as an important risk factor for chronic kidney diseases (CKDs) [2–4]. In 1974, Weisinger et al. [5] firstly reported that massive obese patients developed nephrotic-range proteinuria. Subsequent studies confirmed that obesity could induce renal injury, namely, obesity-related glomerulopathy (ORG) [6–8]. A large-scale clinicopathologic study including 6818 renal biopsies from 1986 to 2000 revealed a progressive increase in biopsy incidence of ORG from 0.2% in 1986–1990 to 2.0% in 1996–2000 [8]. The tenfold increase in incidence of ORG over 15 years suggests a newly emerging epidemic [8].

The clinical characteristics of subjects with ORG typically manifest with nephrotic or subnephrotic proteinuria, accompanied by renal insufficiency [8–10]. Histologically, ORG presents as focal segmental glomerulosclerosis (FSGS) and glomerular hypertrophy or glomerular hypertrophy alone and relatively decreased podocyte density and number and mild foot process fusion [8, 11, 12]. Clinically, it is distinguished from idiopathic FSGS (I-FSGS) by its lower incidence of nephrotic syndrome, more benign course, and slower progression of proteinuria and renal failure [8, 11].

ORG is an increasing cause of end-stage renal disease (ESRD). The pathophysiology of ORG remains incompletely understood. Potential mechanisms by which obesity affects renal physiology include altered renal hemodynamics, insulin resistance, hyperlipidemia, activation of renin-angiotensin-aldosterone system (RAAS), inflammation, and oxidative stress. Increases in both glomerular filtration rate (GFR) and renal plasma flow (RPF) were observed in obese subjects and animals [13, 14]. This likely occurs because of afferent arteriolar dilation as a result of proximal salt reabsorption, coupled with efferent renal arteriolar vasoconstriction as a result of elevated angiotensin II (AngII) [15]. These effects may contribute to hyperfiltration, glomerulomegaly, and later focal glomerulosclerosis [8, 9]. Insulin
resistance can raise the transcapillary pressure gradient and cause hydrostatic pressure and hyperfiltration by reducing norepinephrine-induced efferent arteriolar constriction [16], leading to glomerular hypertrophy and sclerosis. Hyperinsulinemia also has been shown to stimulate the synthesis of growth factors such as insulin-like growth factor-(IGF-) 1 and IGF-2 and transforming growth factor-β1(TGF-β1), which accelerate production of extracellular matrix and promote glomerular hypertrophy and sclerosis [17, 18]. Hyperlipidemia may promote glomerulosclerosis through mechanisms that involve engagement of low-density lipoprotein receptors on mesangial cells, direct podocyte toxicity, oxidative cellular injury, macrophage chemotaxis, and increase renal expression of sterol regulatory element-binding proteins (SREBP-1 and SREBP-2), resulting in the renal accumulation of cholesterol and triglycerides and together with significant renal increase of fibrogenic cytokines [19, 20]. Adipose tissue is the major source of the components of RAAS. Obese subjects usually have increases in plasma renin activity, angiotensinogen, angiotensin-converting enzyme activity, and circulating AngII, which trigger or promote renal damage by renal hemodynamic changes and nonhemodynamic pathways such as hyperinsulinemia, oxidative stress, and inflammation [21–23]. Inflammatory abnormalities and oxidative stress are characteristic findings of obesity and play important roles in the renal damage associated with obesity, which will be discussed in detail in the following.

2. Inflammation in Obesity-Related Glomerulopathy

Recent studies have demonstrated that obesity causes chronic low-grade systemic inflammation and thus contributes to the development of systemic metabolic dysfunction that is associated with obesity-related disorders and renal disease [24–27]. Levels of some inflammatory markers and cytokines such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and macrophage migration inhibitory factor (MIF) are elevated, whereas concentrations of adiponectin, a protein hormone that exerts anti-inflammatory activities, are reduced in obesity [28–34].

2.1. Leptin. Leptin is a 16-kDa-peptide hormone encoded by obese (ob) gene that is mainly produced by adipose tissue. Leptin serves as a regulator of energy balance by binding to the full-length leptin receptors—obese receptor b (Ob-Rb) in the hypothalamus, leading to reduction in food intake and elevation in temperature and energy expenditure [35]. Leptin receptors can be classified as secreted-forms (Ob-Rec), short-forms (ob-Ra, c, d, and f) mainly expressed in peripheral tissue, and long-forms (ob-Rb) predominantly expressed in hypothalamus. The kidney expresses abundant concentrations of the truncated isoform of the leptin receptor Ob-Ra, but only a small amount of the full-length receptor Ob-Rb [36]. Leptin production is associated with increased size of adipocytes and is positively correlated with the body mass index (BMI) [37]. Increased circulating leptin, a marker of leptin resistance, is common in obesity. Obesity-induced leptin resistance injures numerous peripheral tissues including kidney, liver, myocardium, and vasculature [36, 38]. Leptin results in the development of renal disease by binding to its specific receptors in renal endothelial cells and mesangial cells. In glomerular endothelial cells, leptin stimulates cellular proliferation, TGF-β1 synthesis, and type IV collagen production [36, 39]. In mesangial cells, leptin upregulates synthesis of the TGF-β type II receptor, but not TGF-β1, and stimulates glucose transport and type I collagen production through signal transduction pathways involving phosphatidylinositol-3-kinase [40]. Leptin also stimulates hypertrophy, but not proliferation in cultured rat mesangial cells [41]. However, both those cell types increase their expression of extracellular matrix in response to leptin. Transgenic mice with leptin overexpression demonstrated an increase in collagen type IV and fibronectin mRNA in the kidney [41]. Leptin is involved in the development of glomerulosclerosis through a paracrine TGF-β pathway (between glomerular endothelial and mesangial cells) that promotes the deposition of extracellular matrix, proteinuria, and, eventually, glomerulosclerosis [39]. Infusion of leptin into normal rats for 3 weeks fosters the development of focal glomerulosclerosis and proteinuria [36].

Leptin also has proinflammatory actions through its interaction with mediators of innate and adaptive immunity and CRP [38]. Leptin regulates components of innate and adaptive immunity, including T lymphocytes and monocytes/macrophages [42, 43]. Central leptin administration in ob/ob mice accelerates renal macrophage infiltration through the melanocortin system [44]. Leptin stimulates central T-cell production and a peripheral shift in favor of T helper (Th) 1 adaptive immune responses (proinflammatory) as opposed to Th2 responses (anti-inflammatory) [38]. Leptin has been shown to modulate adaptive immunity by enhancing T-cell survival and stimulating production of proinflammatory cytokines such as IFN-γ and IL-2 [45]. Leptin also has structural and functional resemblance to proinflammatory cytokines, such as IL-6 [42], and may modulate CRP, a leptin-interacting protein [46].

Therefore, these direct and indirect effects of leptin on the kidney, including stimulating cellular proliferation and hypertrophy, increasing extracellular matrix expression, and exhibiting proinflammatory activities, may partially explain obesity-related kidney disease.

2.2. Adiponectin. Adiponectin is a 30 kDa adipocyte-derived protein hormone encoded by the adipose most abundant gene transcript 1 (APM1), [47] which plays a role in the suppression of inflammation-associated metabolic disorders. Two distinct adiponectin receptors, AdipoR1 and R2, have been cloned [48]. AdipoR1 is expressed ubiquitously while AdipoR2 is most abundant in the liver. Adiponectin is highly abundant in human serum, but its level is decreased in most obese animal and human subjects, particularly in those with visceral obesity [49–51]. Recent clinical studies show a negative association of adiponectin in obese patients, [52, 53] suggesting that adiponectin may play a key role in the
development of obesity-related albuminuria and alteration of renal function.

Studies with the adiponectin knockout mouse provide evidence that adiponectin can regulate podocyte function and thus contribute to the initial development of albuminuria [37, 53]. Sharma et al. showed that knockout of adiponectin in mice increased albuminuria and caused fusion of podocyte foot processes [53]. In cell culture studies with podocytes, incubation with adiponectin potently decreased permeability to albumin, induced translocation of zona occludens-1 (ZO-1) to the plasma membrane, and reduced the renal predominant NADPH oxidase Nox 4, largely via a 5′-AMP-activated-protein kinase- (AMPK-) dependent pathway. Treatment of the adiponectin knockout mice with exogenous adiponectin was able to decrease albuminuria and improve podocyte morphology [53]. Chronic hyperadiponectinemia significantly alleviated the progression of proteinuria in early-stage diabetic nephropathy by several mechanisms. It led to an increase in nephrin expression, improvement of the endothelial dysfunction due to decreases in endothelin 1 (ET-1) and plasminogen activator inhibitor 1 (PAI-1), and an increase in endothelial nitric oxide synthase (eNOS) expression in the renal cortex [54].

Recent studies suggest that adiponectin exerts anti-inflammatory effects by suppressing TNF-α-induced activation of nuclear factor-κB (NF-κB) in human aortic endothelial cells and aortic smooth muscle cells through inhibition of IκB phosphorylation [55, 56] and inhibition of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) expression [57], thereby reducing monocyte adhesion and macrophage-induced cytokine production [58] and CRP expression in human adipose tissue [59]. Human adipose tissue expressed CRP, which was negatively correlated with adiponectin expression in adipose tissue [59]. Low levels of adiponectin are associated with higher levels of highly-sensitive C-reactive protein (hs-CRP) and IL-6 [49], two inflammatory mediators that are involved in the initiation and progression of atherosclerosis and renal disease. Therefore, hypoadiponectinemia contributes to development of a low-grade systemic chronic inflammation state, suggesting that hypoadiponectinemia may play a causative role in the systemic and vascular inflammation commonly found in obesity and obesity-related disorders, including renal injury, through its proinflammatory effects.

2.3. Resistin. Resistin, also known as adipocyte-specific secretory factor (ADSF) or as found in inflammatory zone (Fizz), is a cysteine-rich 12.5-kDa polypeptide that belongs to a small family called resistin-like molecules (RELMs) [60, 61]. In rodents, resistin is secreted from white adipocytes [62, 63]. In human, it is produced largely by macrophages and expressed in adipose tissue predominantly by nonadipocyte resident inflammatory cells [64–66]. Current evidence suggests that resistin has been variably associated with obesity, insulin resistance, inflammation, and renal dysfunction. Resistin levels are elevated in both genetic and diet-induced animal models of obesity [62, 67]. Studies of obese subjects have frequently noted higher serum levels of resistin as well as direct correlations between resistin level and adiposity as measured by BMI [68, 69]. There has been a link between circulating resistin and low-grade inflammation that accompany obesity [70]. Resistin is associated with elevated CRP and white blood cells, suggesting that the role of resistin may be a component of obesity-related inflammation [70]. It has recently been found that resistin involves in the regulation of proinflammatory cytokine expression. Resistin strongly upregulates IL-6 and TNF-α in human peripheral blood mononuclear cells (PBMCs) via NF-κB pathway [71]. Human resistin enhanced secretion of proinflammatory cytokines, TNF-α and IL-12 in macrophages by NF-κB-dependent pathway [72]. Studies also show that increased levels of resistin in patients with CKD are associated with declined renal function and inflammation [73, 74]. This suggests that resistin may play an important role in obesity and obesity-associated disease by triggering the release of other proinflammatory cytokines.

2.4. Inflammatory Markers. A growing body of evidence indicates that obesity-related glomerulopathy is associated with upregulation of inflammatory mediators [75]. Obesity leads to adipose tissue macrophage infiltration in white adipose tissue and increased levels in proinflammatory cytokines. Several inflammatory mediators released from adipocytes and macrophages, such as TNF-α, IL-6, IL-1β, CRP, monocyte chemotactrant protein 1 (MCP-1), PAI-1, and MIF, contribute to a low level of chronic inflammatory state in obesity and may be responsible for renal injury in obesity-associated glomerulopathy. An emerging pattern of gene expression was observed in adipose tissue in mice fed high-fat-fed diets, indicating a shift toward global upregulation of inflammatory genes, including TNF-α, IL-6, and MCP-1 [76].

TNF-α, a proinflammatory cytokine, is predominantly produced by macrophages infiltrating adipose tissue [77, 78] and can also be produced by the kidney [79]. This cytokine is involved in the genesis of inflammation and contributes to obesity-associated insulin resistance [80–82]. Within the kidney, AngII, advanced glycation end-products (AGEs), and oxidized low-density lipoprotein (LDL) can stimulate TNF-α synthesis from renal cells to initiate local damage [83–86]. A recent study demonstrates that TNF-α reduces the expression of Klotho, a protein expressed by renal cells, through an NF-κB-dependent mechanism, which contribute to renal injury [87]. TNF-α enhances the expression of PAI-1 in human adipose tissue and plasma PAI-1 levels in obesity subjects and is responsible for reduced fibrinolysis and also a component of extracellular matrix, leading to renal fibrosis and terminal renal failure [88–90]. TNF-α also has been shown to induce the expression of MCP-1 via p38 mitogen-activated protein kinase (MAPK) signaling pathway in renal mesangial cells [91]. MCP-1, a key regulator in recruiting monocytes to the glomeruli, may also contribute to renal damage at a later stage of kidney disease in obesity.

IL-6 is another important proinflammatory mediator systemically secreted from adipose tissue and locally produced in the kidney. Studies have demonstrated the positive relationship between BMI and plasma IL-6 concentrations [92–94]. Studies also suggest that IL-6 plays a key role in
3. Oxidative Stress in Obesity-Related Glomerulopathy

3.1. Obesity and Oxidative Stress. Oxidative stress is caused by an imbalance between increased production of reactive oxygen species (ROS) and/or reduced antioxidant activity, leading to oxidative damage to cells or tissue including lipids, proteins and DNA. It is known that oxidative stress is involved in pathological processes of various diseases, such as cancer, diabetes mellitus, hypertension, and cardiovascular disease. Studies have suggested that obesity is associated with increased oxidative stress [103, 104]. Analysis of oxidative markers in obesity subjects indicates that oxidative damage is associated with increased BMI and percentage of body fat [105, 106]. Conversely, parameters of antioxidant capacity are inversely related to the amount of body fat and central obesity [107, 108]. The possible mechanisms of obesity-related oxidative stress include increased oxygen consumption and subsequent production of free radicals derived from the increase in mitochondrial respiration, diminished antioxidant capacity, fatty acid oxidation, lipid oxidizability, and cell injury causing increased rates of free radical formation [104, 109, 110]. It is also reported that the increase in obesity-associated oxidative stress is due to the presence of excessive adipose tissue accumulation [111]. Accumulated adipose tissue generates an immune response leading to the secretion of proinflammatory cytokines, including TNF-α, IL-1, and IL-6, which lead to increased generation of ROS. Excessive fat accumulation also stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, which contributes to ROS production [112]. ROS, in return, augmented the expressions of NADPH oxidase (NOX) subunits, including NOX4 and PU.1 in adipocytes, establishing a vicious cycle that augments oxidative stress in white adipose tissue and blood [112].

3.2. Oxidative Stress and Inflammation. Oxidative stress in adipocyte seems to be responsible for the low-grade proinflammatory state commonly observed in obesity [113, 114]. Oxidative stress is increasingly viewed as a major upstream component in cell-signaling cascades involved in inflammatory responses, stimulating the expression of proinflammatory cytokines. ROS activate redox-sensitive transcription factors, particularly NF-κB, inducing the release of proinflammatory cytokines and the expression of adhesion molecules and growth factors, including TNF-α, IL-6, IL-1β, TGF-β1, connective tissue growth factor, IGF-1, platelet-derived growth factor, and VCAM-1 [115, 116]. H$_2$O$_2$ stimulates IL-4 and IL-6 gene expression and cytokine secretion by an apurinic/apyrimidinic-endonuclease/redox-factor-1- (APE/Ref-1-) dependent pathway [117]. ROS increased the expression levels of PAI-1, IL-6, and MCP-1 through NADPH oxidase pathway [112]. Oxidized high-density lipoprotein (HDL) enhances proinflammatory properties such as TNF-α and MCP-1 in renal mesangial cells partly via CD36 and LDL receptor-1 and via MAPK and NF-κB pathways [118]. Oxidized LDL can stimulate TNF-α synthesis from renal cells and initiate local effects of renal damage [85]. Increased ROS production and MCP-1 secretion from accumulated fat may cause infiltration of macrophages and inflammation in adipose tissue of obesity [112]. Moreover, enhanced macrophage migration induces the release of proinflammatory cytokines, which further stimulates the generation of ROS [119, 120]. Therefore, oxidative-stress-induced cytokine production is likely to further increase oxidative stress levels, setting a vicious cycle [121] that may promote the progression of kidney damage in obesity.

3.3. Oxidative Stress Leads to Renal Injury in Obesity. Oxidative stress has been commonly identified in obesity-related renal diseases and may be the mechanism underlying the initiation or progression of renal injury in obesity [122, 123]. Previous studies suggest that oxidative stress triggers, at an early age, the onset of kidney lesions and functional impairment in Zucker obese (ZO) fa/fa rats, a good model of obesity-related renal disease, in absence of hyperglycaemia, hypertension, and inflammation [124]. ROS play a crucial role in mediating renal injury [125–127]. ROS are highly reactive molecules that oxidize lipids and proteins, cause cellular injury, and promote glomerular and renal tubule injury and associated proteinuria [128].

ROS are produced by various cells, such as vascular cells, inflammatory cells, and renal cells, and have distinct function on different types of cells, such as endothelial dysfunction, inflammatory gene expression, and renal tubule ion transport. A major source for vascular and renal ROS is a Nox family of nonphagocytic NAD(P)H oxidases, including the prototypic Nox2 homolog-based NAD(P)H oxidase, as...
well as other NAD(P)H oxidases, such as Nox1 and Nox4 [129]. Numerous reports indicate that within the kidney, NAD(P)H oxidase, an enzyme that produces superoxide (O$_2^−$) by transferring electrons from NADH/NADPH to molecular oxygen and thereby forming O$_2^−$, H+, and NAD$^+/NADP^+$, is capable of modulating renal epithelial ion transport [130, 131]. NAP(D)H oxidase-derived ROS can alter renal pressure natriuresis and blood pressure regulation through its effects on renal hemodynamics and renal tubular sodium transport. Recent data suggest that NAPD+H oxidase-mediated oxidative injury to the proximal tubule, like that seen in the glomerulus, contributes to proteinuria in insulin-resistant states [128].

Oxidative stress also plays an important role in the pathogenesis of renal damage through its effects on vascular biology. ROS are generated by all types of vascular cells, including endothelial, smooth muscle, and adventitial cells. ROS influence vascular cell growth, migration, proliferation, and activation [132, 133]. Physiologically, ROS can mediate cellular function, receptor signals, and immune responses on vascular cells. In pathophysiological condition, ROS contribute to progressive vascular dysfunction and remodeling through oxidative damage caused by decreased nitric oxide (NO) bioavailability, impaired endothelium-dependent vasodilatation and endothelial cell growth, apoptosis or anoikis, endothelial cell migration, and activation of adhesion molecules and inflammatory reactions [134, 135].

4. Potential Interventions to Prevent Renal Injury

4.1. Anti-Inflammation Therapy. Obesity accelerates the progression of renal injury, associated with augmented inflammation in adipose and kidney tissues [136]. Anti-inflammation therapy might be a potential treatment for renal damage. IL-6 is a key inflammatory molecule in renal diseases. Studies in IL-6 transgenic mice suggested that high concentrations of IL-6 contribute to development of renal injury [137]. Treatment with anti-IL-6 receptor antibody MR16-1 prevented progression of proteinuria, renal lipid deposit, and the mesangial cell proliferation in hypercholesterolemia-induced renal injury [97]. TNF-α is another important proinflammatory cytokine in the development of renal diseases. Inhibition of TNF-α by etanercept, a TNF-α antagonist, also decreased blood pressure and protected the kidney through reduction of renal NF-κB, oxidative stress, and inflammation [138]. TNF-α blockade increases renal Cyp2e23 expression and slows the progression of renal damage in salt-sensitive hypertension [139]. TNF-α inhibition also reduces renal injury in deoxycorticosterone-acetate (DOCA-) salt hypertensive rats via suppression of renal cortical NF-κB activity [140]. Adiponectin is an adipose-secreted hormone with anti-inflammatory properties. Treatment of adiponectin-knockout mice with adenovirus-mediated adiponectin results in amelioration of albuminuria, glomerular hypertrophy, and tubulointerstitial fibrosis and reduces the elevated levels of VCAM-1, MCP-1, TNF-α, TGF-β1, collagen type I/III, and NADPH oxidase components [141]. Adiponectin prevents glomerular and tubulointerstitial injury through modulating inflammation and oxidative stress [141].

Excessive fat accumulation contributes to macrophage infiltration in adipose tissue and increased production of proinflammatory cytokines, such as TNF-α, IL-8, and IL-6 [142–145]. Consequently, it is possible that weight loss may be a potential method to reduce inflammation. Evidence indicates that weight loss induced by nutritional intervention or gastric surgery markedly improves the systemic and adipose tissue inflammatory states linked to obesity [143, 146, 147]. Studies by gene profiling analysis have shown that caloric restriction-induced weight reduction leads to the regulation of a wide variety of inflammation-related molecules in human adipose tissue [148]. Weight loss globally improves the inflammatory profile of obese subjects through a decrease of proinflammatory factors and an increase of anti-inflammatory molecules in white adipose tissue [148]. Roux-en-Y-Gastric-Bypass- (RYGB-) induced weight loss has been shown to reduce MCP-1, IL-18, IL-6, and TNF-α concentrations [149, 150]. A longer-term weight reduction induced by RYGB in corpulence also prevails in regulating circulating cytokine concentrations [151]. Weight loss also ameliorates the low-grade inflammation state that leads to glomerular dysfunction in obesity. In morbidly obese individuals with glomerular hyperfiltration, weight loss by surgical interventions normalizes glomerular filtration rate (GFR) and reduces blood pressure and microalbuminuria [152]. Weight loss improves renal function as shown by reduced levels of serum creatinine and improved creatinine clearance [153].

4.2. Antioxidant Intervention. Since ROS play a key role in the pathogenesis of renal injury such as glomerulosclerosis and tubulointerstitial fibrosis, approaches to reduce oxidative stress by antioxidants supplementation, nutritional and surgical interventions may have renoprotective effects. Garcia protects against obesity-induced nephropathy by attenuating oxidative stress through reduced lipid peroxidation and levels of oxidized LDL [154]. The obese Zucker rat is a good model for studying obesity–related kidney disease because it develops proteinuria, glomerular hypertrophy, and focal segmental glomerulosclerosis [155–157]. Using these rats, it has been demonstrated that nephropathy is associated with oxidative stress, and supplementation with an antioxidant ebselen improved kidney damage by ameliorating proteinuria and renal focal and segmental sclerosis [158]. Chronic ebselen therapy also improved vasculopathy with lipid deposits, tubulointerstitial scarring, and inflammation [158]. Other antioxidants also have renoprotective effects. For example, administration of grape seed proanthocyanidin extract (GSPE), an efficient phytochemical antioxidant, can protect against the nephrotoxicity effects induced by cisplatin and gentamicin [159, 160] and reverse experimental myoglobinuric acute renal failure [161]. Quercetin, a flavonoid that exhibits antioxidant properties in many diseases, could also protect the rat kidney against lead-induced injury and improve renal function [162]. Thus, antioxidants may be a potential therapeutic to prevent the renal damage in ORG.

Nutritional and surgical interventions are additional approaches to reduce oxidative stress and prevent kidney injury...
in obesity. Caloric restriction and protein restriction reduce free radicals and ROS formation and inhibit accumulation of oxidative biomarkers in animal models [163]. In genetically obese animals, diet restriction can prevent or greatly delay the onset of specific degenerative lesions, in particular glomerulonephritis associated with obesity [164]. Since adipose tissue mass in obesity contributes to oxidative stress, bariatric surgery-induced weight loss also results in decreasing systemic oxidative stress in adiposity [111]. Weight loss induced by diet restriction or bariatric surgery not only improves inflammation state but also reduces oxidative stress state in obesity, which may protect renal function in obesity-related glomerulopathy.

5. Conclusion

Obesity causes chronic low-grade inflammation and systemic and local oxidative stress, which may play a pivotal role in the initiation or progression of obesity-associated glomerulopathy. Elevated inflammation in obesity is the result of the production of adipokines and increased inflammatory cytokines and decreased anti-inflammatory factors. Oxidative stress is triggered by an imbalance between increased production of ROS and/or reduced antioxidant activity. Both inflammation and oxidative stress induce damage to renal tubule and glomerulus and result in endothelial dysfunction in the kidney. Therefore, anti-inflammation and antioxidant interventions may be the potential therapies to prevent and treat obesity-related renal diseases.

References


Review Article

Reactive Oxygen Species Modulation of Na/K-ATPase Regulates Fibrosis and Renal Proximal Tubular Sodium Handling

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Received 7 October 2011; Accepted 7 November 2011

Academic Editor: Ayse Balat

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The Na/K-ATPase is the primary force regulating renal sodium handling and plays a key role in both ion homeostasis and blood pressure regulation. Recently, cardiotonic steroids (CTS)-mediated Na/K-ATPase signaling has been shown to regulate fibrosis, renal proximal tubule (RPT) sodium reabsorption, and experimental Dahl salt-sensitive hypertension in response to a high-salt diet. Reactive oxygen species (ROS) are an important modulator of nephron ion transport. As there is limited knowledge regarding the role of ROS-mediated fibrosis and RPT sodium reabsorption through the Na/K-ATPase, the focus of this review is to examine the possible role of ROS in the regulation of Na/K-ATPase activity, its signaling, fibrosis, and RPT sodium reabsorption.

1. Introduction

According to the American Heart Association (AHA), over 70 million people in the United States aged 20 and older have high blood pressure (BP). The cause of 90–95 percent of the cases of high BP is unknown, yet in the last decade the associated morbidity and mortality from high BP has increased precipitously. In the 2008 AHA Scientific Statement [1], excessive dietary salt intake is listed as one of the major lifestyle factors which significantly contributes to the development of hypertension and tends to be more pronounced in typical salt-sensitive patients. Modest dietary salt restriction and diuretic therapy, therefore, are recommended for treatment of resistant hypertension, especially in the salt-sensitive subgroup [1, 2]. Renal sodium handling is a key determinant of long-term BP regulation [3]. The relationship between dietary sodium, salt sensitivity, and BP has been established on an epidemiological and clinical basis. It is estimated that hypertension affects 25% to 35% of the world population aged 18 and older [4], and more hypertensive subjects (~50%) are significantly salt sensitive than normotensive subjects (~25%) [5]. In the DASH-Sodium clinical trial, BP reduction was correlated with sodium restriction in the salt-sensitive subjects regardless of diet [6]. Interestingly, animal renal cross-transplantation experiments [7–10] and studies of human renal transplantation [11] demonstrate that BP levels “travel with the donor’s kidney,” providing compelling evidence for the role of renal function in the pathogenesis of hypertension. In clinical and experimental models, renal proximal tubule (RPT) sodium handling accounts for over 60% reabsorption of filtered sodium and is an independent determinant of BP response to salt intake, playing a critical role in the pathogenesis of salt-sensitive hypertension. Recently, accumulating data indicate that cardiotonic steroids (CTS) signaling through the Na/K-ATPase contribute to RPT sodium handling and salt sensitivity [12–18].

2. CTS and Na/K-ATPase Signaling

CTS (also known as endogenous digitalis-like substances) include plant-derived digitalis drugs, such as digoxin and ouabain, and vertebrate-derived aglycones such as bufalin and marinobufagenin (MBG) [16, 18]. Recent studies have identified both ouabain and MBG as endogenous steroids whose production, and secretion are regulated by stimuli including angiotensin II (Ang II) [18–21]. The Na/K-ATPase
belongs to the P-type ATPases family and consists of two noncovalently linked α and β subunits [22–24]. Several α and β isoforms, expressed in a tissue-specific manner, have been identified and functionally characterized [22–25]. The α1 subunit contains multiple structural motifs that interact with soluble, membrane and structural proteins including Src, caveolin-1, phospholipase C-γ, PI3 kinase, IP3 receptor, β1P, calnexin, coflin, and ankyrin [26–36]. Binding to these proteins not only regulates the ion-pumping function of the enzyme, but it also conveys signal-transducing functions to the Na/K-ATPase [18, 32, 37–39]. In LLC-PK1 cells, the Na/K-ATPase α1 subunit and Src form a functional receptor in which the binding of CTS to the α1 subunit activates Src and consequent signaling cascade [39]. The signaling function of the Na/K-ATPase regulates numerous cell functions in various types of organs and cells including cell motility, cell proliferation, cancer, endothelin release, glycogen synthesis, apoptosis, hypertension, intracellular calcium signaling, cardiac hypertrophy, cardiac remodeling, renal remodeling, epithelial cell tight junction, vascular tone homeostasis, and sodium homeostasis [26, 40–59]. The topic of CTS-Na/K-ATPase signaling and its downstream pathophysiological implications has been extensively reviewed in the last few years [12–16, 18, 21, 32, 39, 59–64], and we will not discuss them in detail in this review.

3. CTS and Sodium Homeostasis

Endogenous CTS were first proposed many years ago to function as a natriuretic hormone. Although their pathophysiological significance has been a subject of debate for many years [65], the concept of a natriuretic hormone is supported by the experimental observations in animal models of ouabain-induced natriuresis [66, 67]. Recently, several elegant reports have confirmed this concept with different approaches. Gene replacement studies have unequivocally demonstrated an important role of endogenous CTS in regulation of renal sodium excretion and BP [68, 69]. In transgenic mice expressing ouabain-sensitive Na/K-ATPase α1 subunit, a significant observation is an augmented natriuretic response to both acute salt load and ouabain infusion, indicating that the ouabain-binding site of Na/K-ATPase α1 subunit participates in the natriuretic response to salt load by responding to endogenous ouabain. Moreover, the augmented natriuretic response in the ouabain-sensitive α1 isoform mice can be blocked with administration of an anti-digoxin antibody fragment [68, 69]. In normal male Wistar rats, endogenous circulatory ouabain has physiological roles controlling vasculature tone and sodium homeostasis, showing endogenous ouabain regulates renal sodium excretion in normal animals [70]. A significant inhibition of natriuresis is observed in rats that were passively immunized with anti-ouabain antibody and in rats that were actively immunized with ouabain-albumin antigen, in which endogenous ouabain levels were reduced in both cases. Like ouabain, MBG has both natriuretic and vasoconstrictor effects [12, 71, 72]. High salt intake induced an initial transient stimulation of ouabain and a subsequent progressive increase of MBG both in Dahl salt-sensitive (S) rats and in humans [12, 73]. In Dahl S rats, the increase in natriuresis stimulated by an acute salt loading is prevented by administration of both an anti-ouabain antibody and an anti-MBG antibody [72]. Furthermore, endogenous CTS have also been implied in age-related increases in salt sensitivity of BP in human normotensive subjects [73]. It is estimated that approximately 50% of humans with untreated essential hypertension have significantly elevated levels of endogenous ouabain [74] which is involved in the regulation of vascular tone homeostasis through stimulation of interaction between the Na/K-ATPase and sodium/calcium exchanger [59]. In normotensive human males, both a high salt diet and systematic sodium depletion (by hydrochlorothiazide) significantly increase plasma ouabain concentration [75].

Release of endogenous ouabain from the brain hypothalamus stimulated by a high salt diet leads to inhibition of the Na/K-ATPase activity and central sympathetic activation (reviewed in [76, 77]), which plays a crucial role in the pressure effects of high salt intake in spontaneously hypertensive rats (SHR) and Dahl S rats. In Dahl S rats, elevation in brain ouabain increased MBG secretion from the adrenal cortex, and this effect was blocked by the AT1 receptor antagonist losartan [78, 79]. The data suggest that the observed CTS-induced natriuretic effect might be a result of the combined effects of ouabain and MBG [60]. Furthermore, CTS not only induced hypertension in rats but also caused significant cardiovascular remodeling and natriuresis independent of their effect on BP [49, 51, 70, 80, 81].

4. CTS and Oxidative Stress in Cardiac and Renal Fibrosis

CTS binding to the Na/K-ATPase induces cellular reactive oxygen species (ROS) production and its downstream effects, such as cardiac and renal fibrosis, and these effects can be prevented by ROS scavenging [80, 82–84]. In kidney and heart, the central role of CTS in the development of fibrosis has been demonstrated both in vivo, in the partial (5/6th) nephrectomy model, and in vitro cell culture, including cardiac and renal fibroblasts. 5/6th nephrectomy increases circulating levels of MBG and stimulates cardiac fibrosis in both rat and mouse [80, 84, 85]. Rats subjected to 5/6th nephrectomy develop systemic oxidant stress that is similar to that seen in rats subjected to MBG infusion as evidenced by significant elevation of plasma carbonylated protein. Active immunization against MBG and reduction of circulating levels of MBG by adrenalectomy substantially attenuate 5/6th nephrectomy and MBG infusion-mediated cardiac fibrosis and oxidant stress, an effect that is independent of BP [80, 84]. In primary culture of rat cardiac and human dermal fibroblasts as well as a cell line derived from rat renal fibroblasts, MBG and ouabain stimulate [3H]proline incorporation as well as gene and protein expression of collagen [86]. MBG induced a PLC-dependant translocation of PKC-δ to the nucleus, resulting in the phosphorylation and degradation of transcription factor Friend leukemia integration-1 (Fli-1), a negative
regulator of collagen synthesis [86]. Both CTS-induced Na/K-ATPase signaling and oxidative stress are necessary in the pathogenesis of cardiac and renal fibrosis as evidenced by CTS-stimulated phosphorylation of both Src and MAPK which is effectively blocked not only by ROS scavenging and Src inhibition but also through possible competitive inhibition of CTS binding to Na/K-ATPase by spironolactone and canrenone [80, 84, 87, 88]. MBG infusion causes renal fibrosis mainly in the cortex of the kidney by stimulation of the transcription factor Snail expression and its nuclear localization in the tubular epithelia, which is associated with epithelial-to-mesenchymal transition (EMT) during renal fibrosis [89]. This EMT phenomenon is also demonstrated in LLC-PK1 cells, indicating that MBG may cause damage of renal proximal tubules. CTS-induced fibrosis in heart and kidney might shift the cardiac and renal function curve to favor a higher set point of pressure natriuresis (Figure 1).

5. Na/K-ATPase Signaling and RPT Sodium Handling

It has been postulated for decades that endogenous CTS stimulated by increased sodium intake increases natriuresis and diuresis by directly inhibiting renal tubular Na/K-ATPase to prevent renal reabsorption of filtered sodium [90–92]. There is accumulating evidence supporting this idea under conditions such as high salt intake, chronic renal sodium retention, renal ischemia, uremic cardiomyopathy, and volume expansion in various animal models and human beings [12, 13, 15, 60, 62, 75, 93–107]. Although the direct inhibition of the Na/K-ATPase enzymatic activity and sodium reabsorption in RPTs by CTS has not been validated, recent observations indicate that ligand-mediated RPT sodium reabsorption via Na/K-ATPase/c-Src signaling counterbalances the sodium retention-mediated increases in BP, such as that seen in salt-sensitive hypertension [108–114]. Ouabain, a ligand of the Na/K-ATPase, activates the Na/K-ATPase/c-Src signaling pathway and subsequently redistributes basolateral Na/K-ATPase and apical sodium/hydrogen exchanger isoform 3 (NHE3) in RPTs, leading to reduced RPT sodium reabsorption and increased urinary sodium excretion.

In LLC-PK1 cells, ouabain activates Na/K-ATPase/c-Src signaling pathways and reduces cell surface Na/K-ATPase and NHE3, leading to a significant inhibition of active transcellular \( \Delta^{2}Na^{+} \) flux from the apical to basolateral compartment [108–112]. MBG, an important CTS species, and deproteinated extract of serum (derived from patients with chronic renal failure) also induce Na/K-ATPase endocytosis and inhibition of active transepithelial \( 2^{2}Na^{+} \) flux. However, it is still not clear how ouabain-activated Na/K-ATPase signaling regulates NHE3, since, at concentrations of ouabain used, no significant change in intracellular Na\(^+\) concentration was observed [108]. Interestingly, this phenomenon is either not observed or is much less significant in MDCK cells (a canine renal distal tubule cell line). These in vitro data suggest that CTS-Na/K-ATPase signaling has a profound effect on RPT sodium handling, but not in distal tubules.

Different species of endogenous CTS show differences in the kinetics and tissue actions in response to salt loading in both animal models and in human salt-sensitive hypertensive patients [16, 18, 60, 75, 79, 105]. The Dahl salt-resistant (R) and salt-sensitive (S) strains were developed by selective breeding of the outbred Sprague-Dawley rat strain for resistance or susceptibility to the hypertensive effects of high dietary sodium [115]. RPT sodium handling is a critical determinant of the different BP responses in these strains [7, 116–118], and there is no Na/K-ATPase \( \alpha \) gene (\( \alpha_{1g, e n e} \)) difference between R and S rats [119]. In comparison to Dahl S rats that eliminate excessive sodium mainly through pressure natriuresis at the expense of an elevated systolic BP, a major response to salt loading in the Dahl R rats is a greater reduction in renal sodium reabsorption to eliminate excessive sodium without raising BP. Our recent in vivo observation [114] is in agreement with this hypothesis and our in vitro observations in LLC-PK1 cells. Specifically in isolated RPTs, both a high salt diet and ouabain are able to activate Na/K-ATPase/c-Src signaling pathways, leading to the redistribution of Na/K-ATPase and NHE3 in the Dahl R but not in the S rats. The R rats show significant increases in total urinary sodium excretion and RPT-mediated fractional sodium excretion, without BP elevation [114]. While the BP response to salt loading in R and S rats involves many regulatory factors [117], our data indicate that impairment of the RPT Na/K-ATPase/c-Src signaling contributes to salt sensitivity. Since it failed to confirm the possible difference of Na/K-ATPase \( \alpha \) gene (\( \alpha_{1g, e n e} \)) between R and S rats [119], other factor(s) must be present to prevent activation of Na/K-ATPase/c-Src signaling in the S rats. The possible effect of CTS on RPT Na/K-ATPase signaling and sodium reabsorption is summarized in Figure 2.

The SHR rat is an established model of human essential hypertension with the characteristic of vascular resistance. SHR rat develops hypertension spontaneously at the age of 7–15 weeks, regardless of salt loading, mainly through
increase in peripheral vascular resistance [120] including renal vascular resistance. Interestingly, either reduction of dietary vitamin E or caloric restriction without sodium restriction prevents the development of hypertension [121, 122]. Like Dahl S rats, SHR rats eliminate excessive sodium via a pressure-natriuresis mechanism but have significant lower BP response to a high salt diet and higher systolic BP via a pressure-natriuresis mechanism but have significant lower BP response to a high salt diet and higher systolic BP [123–126]. Most interestingly, comparing to control Wistar-Kyoto (WKY) rats, regulation of the Na/K-ATPase and NHE3 with both aging and oxidative stress have been shown contributed to the development and maintenance of hypertension in the SHR [127–135]. In renal cortical (proximal) tubules, activity and protein levels of NHE-3 are significantly higher in SHR than age-matched WKY rats at all stages during the development and maintenance of hypertension. When NHE3 function is determined by the rate of bicarbonate reabsorption by in vivo stationary microperfusion in RPT, young SHR rats show higher NHE3 activity than adult SHR and WKY rats, and this is accompanied by changes in NHE3 phosphorylation and distribution. In young SHR rats, the RPT Na/K-ATPase activity is significantly higher than in age-matched WKY, which can be prevented by treatment of the diuretic drug hydrochlorothiazide. However, the Na-K-ATPase activity in medullary thick ascending limb, cortical thick ascending limb, and distal tubule is significantly lower in young SHR rats than in age-matched WKY rats. There were no significant differences in Na/K-ATPase activity in these nephron segments in adult SHR and WKY rats, nor in collecting duct segments of young and adult SHR and age-matched WKY rats. Interestingly, however, the Na/K-ATPase a1 subunit gene expression is lower in both young and adult SHR rats than age-matched WKY rats, indicating a posttranscriptional regulation.

Recent study indicates that SHR rats have enhanced renal superoxide generation and NADPH oxidase (NOX) expression in both vascular and renal tissue before and after development of hypertension [136]. Elevated basal level of superoxide inhibits proximal tubule NHE3 activity and fluid reabsorption in SHR rats in comparison to WKY rats. This effect is prevented by the NOX inhibitor apocynin or knockdown of the critical NOX subunit p22phox with small interfering RNA, indicating that increased basal level of superoxide impairs RPT function [137]. Furthermore, in Sprague-Dawley rats, oxidative stress impairs Ang II-mediated regulation of NHE3 [138].

6. Oxidative Stress and Regulation of Na/K-ATPase

Many stimuli including hypoxia and dopamine induce a cell- and tissue-specific endocytosis and exocytosis of Na/K-ATPase and a change in Na/K-ATPase activity [33, 61, 139]. Ouabain-stimulated ROS generation functions as a second messenger in ouabain-activated Na/K-ATPase signaling in isolated rat cardiac myocytes [82, 140–142]. Binding of ouabain to the Na/K-ATPase activates Src kinase, which in turn transactivates EGFR, leading to activation of the Ras-Raf-MEK-ERK pathway [32, 39, 63]. Ras activation leads to the activation of MAPK and increase in [Ca^{2+}]i, which result in opening of mitochondrial ATP-sensitive K+ channels [142] and generation of mitochondrial ROS [82, 141]. ROS subsequently activate NF-kB [141, 143] and slow [Ca^{2+}]i oscillations at nanomolar ouabain concentrations [40]. Additionally, ouabain-induced generation of ROS in neonatal myocytes is antagonized by overexpression of a dominant negative Ras as well as myxothiazol/diphenyleneiodonium, indicating a mitochondrial origin of the Ras-dependent ROS generation [82]. Ouabain also stimulates ROS generation in other cell types [144–146]. Conversely, oxidative stress can activate the Na/K-ATPase signaling. Both a bolus of H_{2}O_{2} and exogenously added glucose oxidase (which generates a sustained low level of H_{2}O_{2} by consuming glucose) activates Na/K-ATPase signaling in cardiac myocytes [140]. Pretreatment with the antioxidant N-acetyl cysteine (NAC) prevents ouabain-Na/K-ATPase signaling and its downstream effects. Moreover, infusion of CTS causes ROS generation and protein oxidation in experimental animals [80, 147].

The redox sensitivity of the Na/K-ATPase was first demonstrated in electric eels with treatment of H_{2}O_{2} [148]. This phenomenon was further observed in a wide range of animal species, tissues, and other species of ROS such as hypochlorous anion, hydroxyl radicals, superoxide, hyperchlorite anions, and singlet oxygen. It has been shown that the Na/K-ATPase in skeletal muscle is redox-sensitive and infusion of NAC attenuated ROS-mediated inhibition of maximal pump activity [149]. In dog kidney, oxidative modification of kidney Na/K-ATPase by H_{2}O_{2} was accompanied by a decrease in the amount of sulfhydryl (SH) groups [150] and, importantly, oxidative modification can result in formation of Na/K-ATPase oligomeric structure [151]. The differences in the number, location, and accessibility of SH groups in Na/K-ATPase isozymes might predict their oxidative stability [152]. Different antioxidant enzymes, natural or synthetic antioxidants, and some inhibitors of oxidase activity can...
attenuate ROS mediated inhibition of Na/K-ATPase activity. ROS are known to inhibit Na/K-ATPase activity in different types of cells as well. Interestingly, the Na/K-ATPase in rat cerebellar granule cells are redox-sensitive with an “optimal redox potential range,” where ROS levels out of this “optimal range” are capable of inhibiting Na/K-ATPase activity [153]. While the Na/K-ATPase does not contain heme groups, it does contain cysteine residues located in α subunit cytosolic loops which may determine the redox sensitivity of the α subunit. The sensitivity of the Na/K-ATPase to redox and oxygen status, the regulatory factors which govern these interactions, and the implied molecular mechanism were recently reviewed [154].

Increases in ROS can oxidize the Na/K-ATPase α/β subunits and its independent regulator FXYD proteins. This oxidation inhibits its activity and promotes its susceptibility to degradation by proteasomal and endosomal/lysosomal proteolytic pathways in different cell types including cardiac myocytes, vascular smooth muscle cells, and RPTs [155–166]. It appears that the oxidized modification of the Na/K-ATPase is a reversible, redox-sensitive modification. However, purified enzyme has also been shown to be irreversibly inhibited upon exposure to hydrogen peroxide, the superoxide anion, and the hydroxyl radical [158]. The regulation of renal Na/K-ATPase α1 by oxidants is not dependent on the ouabain sensitivity of the α1 subunit per se. It appears that the α2 and α3 subunits are more sensitive to oxidants than the α1 subunit, and ouabain-sensitive α1 (canine) and insensitive-α1 (rat) have similar sensitivity to oxidants, suggesting the regulation of α1 by ROS is not species specific. Furthermore, ROS accelerates degradation of the oxidatively damaged Na/K-ATPase and the α2 and α3 subunits, which also appear to be more susceptible to degradation than the α1 subunit [159]. These studies indicate that differential oxidant sensitivities of the Na/K-ATPase subunits are dictated by the primary sequences of different subunits and different subunit compositions of the various tissues may contribute to their relative susceptibilities to oxidant stress. In the RPT cell line originated from WKY rats, cadmium, a ROS generator, stimulates ROS production and causes a toxic oxidative damage of the Na/K-ATPase. Oxidative damage increases Na/K-ATPase degradation through both the proteasomal and endo-/lysosomal proteolytic pathways [163]. In purified renal Na/K-ATPase, peroxynitrite (ONOO−) causes tyrosine nitration and cysteine thiol group modification of the Na/K-ATPase, but only cysteine thiol group modification is implied in the inhibition of the enzyme activity since glutathione is unable to reverse the inhibition [167]. In isolated rat renal RPTs, peroxynitrite and its signaling participates in Ang II-induced regulation of renal Na/K-ATPase activity [168].

Ang II inhibits the Na/K-ATPase via PKC-dependent NOX activation. The dependence of Ang II-induced Na/K-ATPase inhibition on NOX and superoxide, as well as reversible oxidative modification of the Na/K-ATPase, strongly suggests a role for redox signaling [164–166]. In cardiac myocytes and pig kidney, oxidative stress induces glutathionylation of the β1 subunit of the Na/K-ATPase. In purified pig renal Na/K-ATPase, peroxynitrite inhibits the enzymatic activity by stabilization of the enzyme in an E2-prone conformation. At the same time, FXYD proteins reverse oxidative stress-induced inhibition of the Na/K-ATPase by facilitating deglutathionylation of the β1 subunit. Moreover, both tyrosine kinase c-Src and cell membrane structural component lipid rafts, which are critical in Na/K-ATPase/c-Src signaling, are also critical in redox signaling platform formation [169–172]. These observations, along with the fact that c-Src is redox-sensitive [173] and its activation regulates NOX-derived superoxide generation [174], suggests a redox-sensitive Na/K-ATPase/c-Src signaling cascade and its possible role in ROS regulation, although the mechanism is not clear. Our unpublished data suggest that certain basal levels of ROS might be required for the initiation of ouabain-Na/K-ATPase/c-Src signaling. In LLC-PK1 cells, pretreatment with higher concentrations of NAC (5 and 10 mM for 30 min), but not with lower concentration of 1 mM, can prevent ouabain-stimulated c-Src activation and the redistribution of Na/K-ATPase and NHE3. This suggests that ROS may stabilize Na/K-ATPase in a certain conformational status in order to facilitate ouabain binding to the Na/K-ATPase α1 subunit and favor ouabain-Na/K-ATPase/c-Src signaling. Some pertinent questions remain to be resolved, such as how ROS interacts with and influences the Na/K-ATPase/c-Src signaling cascade and whether ouabain-induced ROS boosts the Na/K-ATPase signaling by a positive feedback mechanism and chronically desensitizes the signaling cascade by stimulating Na/K-ATPase/c-Src endocytosis (Figure 2).

7. Oxidative Stress and Regulation of Renal Function

ROS function as important intracellular and extracellular second messengers to modulate many signaling molecules. Physiological concentrations of ROS play an important role in normal redox signaling, while pathological levels of ROS contribute to renal and vascular dysfunction and remodeling through oxidative damage [175–177]. Genetic factor(s) partially contribute to high basal ROS levels and the development of hypertension [178, 179].

Oxidative stress has been shown to regulate BP and sodium handling in various animal models. High salt intake increases oxidative stress, and this has important implications for the regulation of cardiovascular and renal systems. An increase in oxidative stress is both a cause and consequence of hypertension [177, 180–183]. Renal NOX is present in the renal cortex, medulla, and vasculature. NOX, the major source of superoxide in the kidney, is of particular interest because of its prominent expression and implication in pathophysiology. In the kidney, increased oxidative stress influences a number of physiologic processes including renal sodium handling in the proximal tubule [137, 168, 184, 185] and thick ascending limb [186], renal medulla blood flow [183, 187–190], descending vasa recta contraction [191, 192] in addition to interactions with other regulatory systems such as the dopamine signaling pathway [193]. Increased oxidative stress has been shown to contribute to salt sensitivity [194, 195]. In macula densa cells, NOX isoform
Nox2 is responsible for salt-induced superoxide generation, while Nox4 regulates basal ROS [196]. In the medullar thick ascending limb of the loop of Henle, both exogenous and endogenous superoxides stimulate sodium absorption [197]. Also, in this nephron segment, NOX-induced generation of superoxide enhances sodium absorption by reduction of the bioavailability of nitric oxide (NO) to prevent NO-induced reduction of NaCl absorption [198], which contributes to salt-sensitive hypertension observed in Dahl salt-sensitive rats [190].

In PRTs, in particular, increases in ROS inhibit the Na/K-ATPase as well as the apical NHE3 and sodium/glucose cotransporter, in order to promote RPT sodium excretion under certain circumstances [137, 168, 184, 185]. While elevated basal level of superoxide inhibits proximal tubule NHE3 activity and fluid reabsorption in SHR rats in comparison to WKY rats [137], peroxynitrite and its signaling participates in Ang II-induced regulation of renal Na/K-ATPase activity in isolated rat renal RPTs [168]. In the pathogenesis of diabetic nephropathy, a high level of glucose-induced ROS generation induced by stimulation of mitochondrial metabolism and NOX activity in RPT primary cultures leads to inhibition of the expression and activity of the sodium/glucose cotransport system [185]. In male Wistar rats, a high salt diet (3% NaCl for 2 weeks) promotes sodium/water excretion and urinary 8-isoprostane excretion, a marker of oxidative stress, which can be attenuated by treatment with apocynin, an NOX inhibitor. The salt loading leads to increased generation of ROS and a state of oxidative stress in the cortex but not to such a degree in the medulla [199].

RPT sodium and/or fluid absorption in the normal rat is reduced by inhibition of NO synthesis, while NO promotes RPT Na+ and/or fluid reabsorption [200]. In immortalized and freshly isolated RPTs from the WKY and SHR rats, the basal level of membrane NOX activity is greater in SHRs [201]. Moreover, NOX-induced superoxide generation inhibits RPT fluid reabsorption in SHRs [137]. In Sprague-Dawley rats treated with the oxidant L-buthionine sulfoximine, Ang II overstimulates RPT Na/K-ATPase and NOX and leads to increased sodium reabsorption, which is prevented by administration of the superoxide scavenger Tempol [202].

High salt diet, which is well documented for its stimulation of systematic oxidative stress, regulates the activity and distribution of the Na/K-ATPase and NHE3 in different animal models [203–207]. RPT sodium reabsorption significantly affects water and sodium homeostasis by regulating redistribution of ion transporters in response to high salt intake [208]. Regulation of NHE3 activity and distribution as well as RPT fluid reabsorption contribute to the development and maintenance of hypertension in young and adult SHR rats [127, 137, 209].

It has become clear that antioxidant agents such as Tempol and enzymes such as heme oxygenase-1 (HO-1) exhibit a beneficial and protective effect on BP in various animal models of hypertension. As an example, inhibition of HO activity reduces renal medullary blood flow [84, 210, 211], total renal blood flow (RBF) [212], glomerular filtration rate (GFR), and renal production of nitric oxide [213]. Inhibition of HO-1 increases mean arterial pressure in Sprague-Dawley rats [214, 215] and SHR rats [216]. Induction of HO-1 reduces BP in SHR rats [217–220]. The effect of HO-1 on BP is presumably through the carbon monoxide (CO)/HO system and depression of cytochrome p-450-derived 20-HETE. In the Dahl salt-dependent model of systemic hypertension, induction of HO-1 occurred in the vasculature and is accompanied by endothelial dysfunction [221, 222]. In Dahl salt-sensitive rats, a high salt diet increases HO-1 expression and CO generation in aorta and coronary arteries [221, 223], and, in Dahl salt-sensitive rats fed low salt diet, induction of HO-1 expression attenuates oxidative stress and reduces proteinuria and renal injury [224]. However, most studies examining the contribution of HO-1 to BP regulation have focused on the vasculature, that is, pressure-natriuresis and arterial BP, and so the role of HO-1 in RPT-mediated sodium handling is still poorly understood. Nevertheless, increased HO expression in RPTs could result in an increased ability to buffer locally produced oxidants, leading to their neutralization.

The beneficial effect of antioxidants is controversial and not seen in most clinic trials with administration of antioxidants (reviewed in [177, 225]). The Dietary Approaches to Stop Hypertension (DASH) study and subsequent studies have demonstrated that lower BP associated with reduced dietary salt intake may be related to reductions in oxidative stress [6, 226–228]. Interestingly, however, while a combination antioxidant supplement (with an ascorbic acid, synthetic vitamin E, and β-carotene) had no improvement on BP after 5-year treatment [229], another combination antioxidant supplement (zinc, ascorbic acid, α-tocopherol, and β-carotene) did result in a significant reduction in systolic BP [230]. Other studies have also shown that certain antioxidants, such as glutathione and vitamin C, have a blood-pressure-lowering effect [231, 232]. However, antioxidant supplementation may be ineffective or even dangerous [233] due to the possible “over-antioxidant-buffering” effect of excessive antioxidant supplementation. In this scenario, excess antioxidants might become pro-oxidants (by providing H+) if they cannot promptly be reduced by the following antioxidant in the biological antioxidant chain. Thus, it appears that the balance of the ROS status, within a physiological range, may be more important to maintain beneficial ROS signaling.

8. Perspective
Renal sodium handling is a key determinant of blood pressure. ROS status, among others, is an important regulator of vasculature and sodium handling. However, the effect of ROS and Na/K-ATPase, especially of their interaction, on RPT sodium reabsorption has only been explored in a limited fashion. Coordinated regulation of two major ion transporters, the basolateral Na/K-ATPase and the apical NHE3, has been implicated in the counterbalancing of high salt intake (volume expansion) mediated blood pressure increase. The Na/K-ATPase/c-Src signaling regulates this
coordinated regulatory mechanism and impairment of this signaling cascade contributes to experimental Dahl salt-sensitive hypertension. Both the Na/K-ATPase (α and β subunit) and its proximal signaling partner c-Src are redox sensitive, suggesting a redox-sensitive Na/K-ATPase/c-Src signaling complex. However, the mechanisms remain largely to be elucidated since the available data is limited [184, 234]. Nevertheless, some pertinent questions remain to be addressed. In the future, it will be important to explore whether ROS signaling is a link between the Na/K-ATPase/c-Src cascade and NHE3 regulation and how oxidative stress stimulated by high salt and CTS regulates Na/K-ATPase/c-Src signaling in renal sodium handling and fibrosis.

References


