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Commentary

Toxico-pharmacological perspective of the Nrf2-Keap1 defense system against oxidative stress in kidney diseases

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ABSTRACT

Oxidative stress, including the generation of reactive oxygen species (ROS), appears to be responsible for the high incidence of cardiovascular events in patients with chronic kidney disease (CKD), and for the progression of CKD to end-stage renal disease. The processes for oxidative stress include increased generation and decreased elimination of ROS that could be caused by an impaired antioxidant defense system. Nuclear factor-erythroid-2-related factor 2 (Nrf2) helps protect the kidney against oxidative stress by playing a pivotal role in the cooperative induction of genes that encode antioxidant and detoxifying enzymes. Nrf2 is confined to the cytoplasm as an inactive complex bound to a repressor Kelch-like ECH-associated protein 1 (Keap1), which facilitates ubiquitination of Nrf2. Studies using CKD model animals showed that despite stimulated oxidative stress the nuclear Nrf2 level was suppressed, which led to downregulation of the antioxidant enzymes. Hence, deterioration in Nrf2-Keap1 signaling could contribute to the severity of oxidative stress and the progression of CKD. By contrast, acute kidney injury (AKI) induces activation of renal Nrf2. Nrf2 activators or its proteasomal degradation inhibitors enhance nuclear Nrf2 translocation, inducing potential renoprotective actions against CKD and AKI. In both chronic and acute kidney diseases, sulfate-conjugated uremic toxins appear to enhance ROS production when accumulated in renal cells. An intestinal indole adsorbent ameliorates the progression of CKD by decreasing accumulation of indoxyl sulfate. Therapeutic approaches to prevent oxidative stress *via* activation of the Nrf2-Keap1 signaling and/or suppression of uremic toxin-induced ROS production could be effective strategies for maintaining kidney function.

1. Introduction

The incidence of chronic kidney disease (CKD) is increasing in both developed and developing nations. It is generally recognized that many patients with CKD are likely to die of cardiovascular disease (CVD) rather than kidney dysfunction [1]. A cohort study comprising >13,000 elderly patients revealed that an increase in the incidence of cardiovascular events could, in part, be related to the fact that patients with kidney disease are less likely to receive preventive treatments against CVD [2]. However, the mechanisms for the enhanced susceptibility to CVD in CKD patients are not fully clarified. The injured and/or dysfunctional kidney-specific risk factors such as endothelial dysfunction, inflammation, oxidative stress, anemia, proteinuria and changes in vitamin D metabolism have been suggested to play a pathophysiological role not only in CVD but also in further progression of CKD [1]. Among these factors, oxidative stress has attracted a great deal of interest from researchers. Oxidative stress appears to increase in the serum of CKD patients because of increased oxidant activity as well as a reduced antioxidant defense system, which is accompanied by kidney dysfunction and/or severe cardiorenal syndrome [3–6].

A transcription nuclear factor erythroid 2-related factor 2 (Nrf2) is characterized as "an oxidative stress-sensing guarding regulator" of more than 200 cytoprotective genes encoding

Abbreviations: ADMA, asymmetric dimethylarginine; AGE, advanced glycation end product; AKI, acute kidney injury; CKD, chronic kidney disease; CVD, cardiovascular disease; ERK, extracellular signal-regulated kinase; GLC, glutamate cysteine ligase; GSH, glutathione; GST, GSH S-transferase; HO-1, heme oxygenase-1; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; MCP-1, monocyte chemoattractant protein-1; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NF-κB, nuclear factor-κB; NQO1, NADPH quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; PPAR-γ, peroxisome proliferator-activated receptor-γ ROSreactive oxygen species; SOD, superoxide dismutase.

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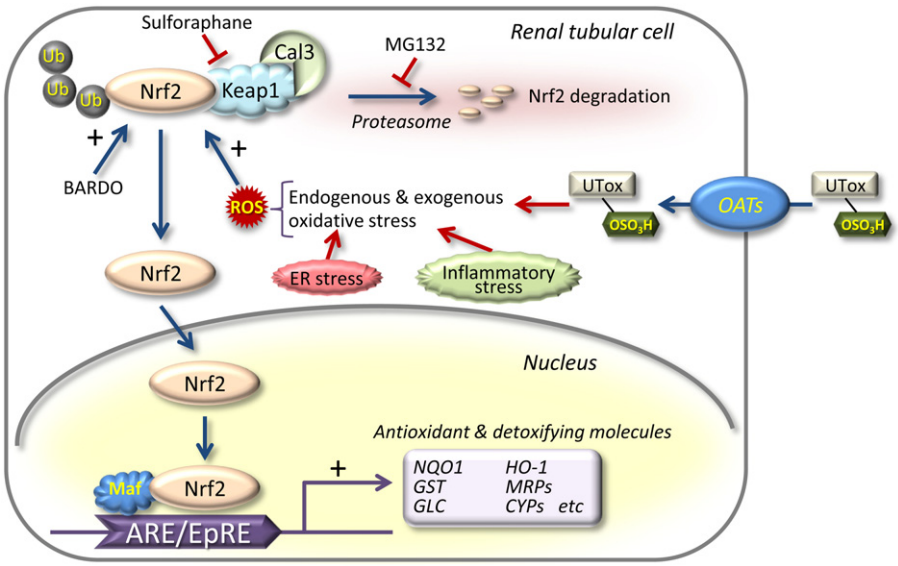

Fig. 1. Schematic representation of the Nrf2-Keap 1 defense pathway in renal tubular cells. Steady state level of Nrf2 in cytoplasm is regulated primarily by modulation of its continuous proteasome degradation following ubiquitination (Ub). Non-toxic concentrations of MG132 inhibit proteasomal degradation of Nrf2 and stimulate the translocation of Nrf2 into the nucleus. Sulforaphane potently activates Nrf2 by modifying Keap1 cysteine residues. Under stress-free conditions, Nrf2 is stably bound in the cytoplasm to Keap1, an E3 ubiquitin ligase substrate adaptor targeting Nrf2 for degradation. Under stressful conditions with endogenous or exogenous ROS evoked by ER (endoplasmic reticulum) stress, inflammatory stress and uremic toxins, conformational changes in Keap1 release Nrf2, increasing half-life of Nrf2 to translocate into the nucleus where it binds to ARE/EpRE (antioxidant/electrophile responsive element with a modulator Maf). After binding to ARE, Nrf2 induces expression of a multitude of antioxidant and detoxifying molecules including NQO1, GST, GLC, HO-1, and MRPs. Bardoxolone methyl (BARDO) activates Nrf2, thereby inducing the transcription of genes that reduce oxidative stress. Sulfate-conjugated uremic toxins (Utox-OSO_3H), such as indoxyl sulfate and p-cresyl sulfate, are accumulated in the cytoplasm *via* OATs, basolateral membrane organic anion transporters OAT1 and OAT3, inducing production of oxidative stress.

proteins that neutralize or detoxify both endogenous metabolites and environmental toxins [7–9]. Nrf2 appears to function when released from its repressive redox-sensitive companion protein Keap1 (Kelch-like ECH-associated protein 1) by sensing cytoplasmic oxidative stress or some chemical agents [8–10] (Fig. 1). After translocation into the nucleus, Nrf2 stimulates transcription of genes encoding detoxifying and antioxidant enzymes, such as NADPH (nicotinamide adenine dinucleotide phosphate) quinone oxidoreductase1 (NQO1), GSH S-transferase (GST), heme oxygenase-1 (HO-1), glutamate cysteine ligase (GLC) and peroxiredoxin I, GSH peroxidase, which contribute to cellular protection by removing reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide and hydroxyl radicals [11]. Although the principal role of the Nrf2-Keap1 defense system in renal ROS production has been well characterized, its toxico-pharmacological role and regulation in "oxidative stress management" of CKD situation are not fully elucidated. Alternatively, ischemic acute kidney injury (AKI) remains a major frequent clinical problem, as AKI aggravates acute mortality and results in permanent and progressive kidney disease, *i.e.*, CKD. In ischemia–reperfusion-induced AKI model animals, ROS appeared to enhance both endothelial and renal tubular injuries [12]. In murine models of AKI, bardoxolone methyl, an orally-available first-in-class synthetic triterpenoid (also known as "RTA 402" or "CDDO-methyl ester"), alleviated functional and structural kidney injuries in association with activation of Nrf2 in glomerular endothelium, cortical peritubular capillaries and renal tubules [13]. Therefore, the Nrf2-Keap1 defense system has been suggested to play a pivotal guardian role in protection of kidneys against diverse oxidative stress generated in both chronic and acute kidney injuries through activating potent antioxidant tools. In this commentary, possible strategic approaches and perspectives focusing on oxidative stress and the Nrf2-Keap1 defense system to prevent the progression of CKD and CVD are discussed.

2. Oxidative stress and the role of Nrf2 in CKD

2.1. Oxidative stress in CKD

The role of oxidative stress has attracted an increasing attention in the field of CKD, cardiorenal syndrome and their preventive strategies [14,15]. Oxidative stress is provoked by excessive production of free radicals, low antioxidant defense or a combination of these two factors. The consequence of oxidative stress is chemical modifications of biomolecules, resulting in structural and/or functional changes. Oxidative stress is defined as the tissue damage resulting from an imbalance between an excessive generation of oxidant species and insufficient antioxidant defense mechanisms. Several processes appear to be involved in ROS production, including mitochondrial respiration, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidases and uncoupled nitric oxide (NO) synthesis. Oxidative stress and inflammation are common features found in patients with CKD in different disease stages, particularly in patients requiring hemodialysis [14,15]. ROS are considered to be major mediators of cardiovascular events and numerous other physiological complications, in addition to playing a critical role in the progression of CKD. Indeed, CKD patients who generate excessive ROS have an increased risk of morbidity and mortality [15]. Several lines of evidence suggest that CKD is a pro-oxidant state. Specifically (i) oxidation markers of lipid, protein and DNA are increased in the serum of CKD patients; (ii) oxidative markers, such as hypochlorous acid (HOCl)-modified lipoproteins and advanced glycation end products (AGEs), are accumulated in atherosclerotic lesions of CKD patients; (iii) there are numerous defects in the antioxidant defense mechanism, resulting in a decreased elimination clearance of ROS [15]. The defects can be used as indirect markers for oxidative stress, *e.g.*, an increased oxidized to reduced plasma ratio of vitamin C and red blood cell GSH level. Increases in the circulating levels of oxidative markers have been documented