Journal of Applied Life Sciences International
2(1): 22-34, 2015; Article no.JALSI.2015.004

SCIENCEDOMAIN *international*
www.sciencedomain.org

Activation of Nrf2 Restores Klotho Expression and Attenuates Oxidative Stress and Inflammation in CKD

Young Ki Son[1,2], Shu-Man Liu[1], Seyed H Farzaneh[1], Sohrab Nazertehrani[1], Mahyar Khazaeli[1] and Nosratola D. Vaziri[1*]

[1]Division of Nephrology and Hypertension, Department of Medicine, University of California, Irvine (Irvine, CA).
[2]Department of Internal Medicine, Dong-A University College of Medicine, Busan, Korea.

Authors' contributions

This work was carried out in collaboration between all authors. Authors NDV and YKS designed the study and wrote the manuscript, authors SN and MK conducted the animal experiments, authors SML and YKS performed the Western blot and chemical assays and author SHF performed the histological and immunohistochemical analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2015/13745
Editor(s):
(1) Dongpei Li, Department of Neuroscience and and Regenerative Medicine, Institute of Molecular Medicine and Genetics, Georgia Regents University, USA.
Reviewers:
(1) Eli A. Friedman, Downstate Medical Center, USA.
(2) Toru Watanabe, Department of Pediatrics, Niigata City General Hospital, Japan.
(3) Anonymous, Chang Jong Christian University, Taiwan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=683&id=40&aid=6850

Original Research Article

Received 1st September 2014
Accepted 9th October 2014
Published 6th November 2014

ABSTRACT

Background: Chronic kidney disease (CKD) causes klotho deficiency, oxidative stress and inflammation which are common features and major mediators of progression of renal disease. Oxidative stress in CKD is, partly, due to impaired activation of Nrf2, the master regulator of genes encoding antioxidant and cytoprotective molecules. Oxidative stress inhibits klotho expression in the kidney. Given the role of Nrf2 dysfunction in the pathogenesis of oxidative stress in CKD, we hypothesized that treatment with Nrf2 activator, dh404, may restore renal klotho expression and attenuate oxidative stress and inflammation in CKD.
Methods: Male SD rats were subjected to 5/6 nephrectomy (CKD) or sham operation. The CKD rats were randomized to receive dh404 (2mg/kg/day) or vehicle for 12 weeks. At the conclusion of the observation period tail arterial pressure was measured and 24-hr urine was collected. Animals

**Corresponding author: Email: ndvaziri@uci.edu*

were then euthanized and blood and kidneys were harvested.
Results: Compared to the control *group*, untreated CKD rats exhibited marked reduction of klotho abundance in the remnant kidney. This was associated with interstitial inflammation, local and systemic oxidative stress, NF_kB activation, impaired Nrf2 activity and reduced expression of the key Nrf2 target gene products. Administration of dh404 reversed klotho deficiency, restored Nrf2 activity and expression of its key target gene products and attenuated oxidative stress and inflammation and NFkB activity in the renal tissue.
Conclusion: Restoration of Nrf2 activity reversed klotho deficiency and attenuated oxidative stress and inflammation in remnant kidneys of CKD rats.

Keywords: Aging; CKD progression; Antioxidants; cytokines; chemokines.

1. INTRODUCTION

Klotho which is expressed predominantly in the kidney was originally identified as an anti-aging protein. However subsequent studies have unraveled many other biological functions of klotho [1-3]. Renal klotho expression is depressed in rats with hypertension and diabetes and in rats and humans with chronic kidney disease (CKD) [4,5]. Studies in transgenic mice have demonstrated the protective effect of over-expression of klotho against ischemia-reperfusion induced acute kidney injury, immune-mediated glomerulonephritis and chronic kidney disease [6-8]. These observations suggest that impaired klotho expression may play a role in the pathogenesis and progression of CKD.

CKD is invariably associated with oxidative stress and inflammation which contribute to progression of CKD and its cardiovascular and numerous other complications [9-12]. In an earlier study Mitobe and Yoshida [13] found marked down-regulation of klotho expression in the renal tubular epithelial cells exposed to oxidative stress. They further found that over-expression of klotho significantly attenuates oxidative stress-induced injury and apoptosis in these cells. In addition, in vivo studies have demonstrated the association of klotho deficiency with oxidative stress and mitochondrial dysfunction in the kidney and its restoration by attenuation of oxidative stress using N-acetylcystein or AT1 receptor blockade in animals with cyclosporine nephropathy [14,15]. These findings clearly illustrate the causal interconnection between oxidative stress and klotho deficiency and their participation in a vicious circuit in CKD. Accordingly the prevailing CKD-associated oxidative stress suppresses klotho expression and klotho deficiency amplifies oxidative stress and inflammation, events that promote progression of renal disease.

Oxidative stress in CKD is caused by a combination of increased production of reactive oxygen species (ROS) and impaired antioxidant capacity [16-20]. The CKD-associated defect in the antioxidant defense system is due to impaired activation of the nuclear factor-erythroid-2-related factor 2 (Nrf2) [20-24). Nrf2 regulates constitutive expression and coordinated induction of over 250 genes encoding antioxidant and phase 2 detoxifying enzymes and related proteins including superoxide dismutase (SOD) isoforms, catalase, UDP-glucuronosyltransferase, NAD(P)H quinone oxidoreductase-1 (NOQ1), heme oxygenase-1 (HO-1), glutamate cystein ligase, glutathione peroxidase and thioredoxin, among others [25]. Under physiologic condition, the Nrf2-mediated regulation of cellular antioxidant and anti-inflammatory machinery plays an important role in the defense against oxidative stress and inflammation [25,26]. In a series of studies we have found marked reductions of Nrf2 activity (nuclear translocation) and expression of its key target gene products in the kidney and vascular tissues of animals with CKD of diverse etiologies [20-22]. These findings have unraveled the role of impaired Nrf2 activity as a common mediator of oxidative stress and inflammation in CKD and a major player in progression of CKD and the associated complications. This supposition is supported by the salutary effects of natural and synthetic Nrf2 activators in animal models of acute and chronic kidney disease [23, 27-36].

Given the central role of Nrf2 in host defense against oxidative stress and inflammation and their role in promoting klotho depletion, the present study tested the hypothesis that administration of an Nrf2 inducer may restore renal klotho expression in rats with CKD. To this end we compared the effect of long term administration of dh404 (CDDO-dh TFEA), a synthetic triterpenoid with potent Nrf2 inducing

properties [37,38], with placebo in rats with CKD induced by 5/6 nephrectomy.

2. METHODS

2.1 Animals

Eight week old male Sprague-Dawley rats were purchased from Harlan Sprague Dawley Inc (Indianapolis, IN), housed in a temperature- and light-controlled vivarium and fed regular rat chew (Purina Mills, Brentwood, MO) and water ad libitum. The animals were randomly assigned to sham-operated control (CTL, n=6), untreated CKD (n=7) and dh404-treated CKD groups (CKD-Rx, n=8). The CKD groups were subjected to 5/6 nephrectomy by removing the upper and lower thirds of the de-capsulated left kidney, followed by right nephrectomy 7 days later. The control group underwent sham operation. All surgical procedures were performed under general anesthesia using intra-peritoneal injection of ketamine/xylazine. Strict hemostasis and aseptic techniques were used.

The treated group received dh404, 2mg/kg/day, dissolved in sesame oil by gastric gavage for 12 weeks. The untreated CKD and control groups received drug free sesame oil instead. The given dh404 dosage was selected after conducting a series of preliminary dose response experiments using daily doses ranging between 0.5-20 mg/kg. We found significant weight loss, marked increase in proteinuria and worsening of kidney pathology in animals treated at doses between 5-20mg/kg but significant improvements at 1-2mg/kg. Arterial pressure was determined by tail plethysmography (CODA2; Kent Scientific, Torrington, CT). The rats were placed on a heated pad in a climate-controlled room, their tails were placed in the cuff and cuff was inflated and released several times. This procedure was repeated on several days to condition the animals. Thereafter, 3 separate measurements were performed and the mean of the values obtained was used. At the conclusion of the study period animals were placed in metabolic cages for 24-hour urine collections. The rats were then anesthetized with intra-peritoneal administration of Ketamine/Xylazine and euthanized by exsanguinations using cardiac puncture. Kidneys were immediately removed and sections were processed for histological, immunohistological and Western blot analyses. Plasma urea nitrogen and creatinine concentrations, and urinary protein excretion were measured as described in previous studies [10]. Plasma malondialdehyde-thiobarbituric acid (MDA-TBA) was measured, using HPLC, as described earlier [11]. The experimental protocol was approved by Institutional Committee for Animal Care and Use of the University of California, Irvine.

2.2 Histological Examination

Light microscopy was performed in the formalin-fixed sections stained with periodic acid-Schiff and hematoxylin and eosin. Glomerulosclerosis and tubulointerstitial damage were graded using the score index described in our previous studies [39].

2.3 Western Blot Analysis

100mg of kidney cortex was homogenized on ice in lysis buffer containing 10 mM HEPES, pH7.9, 1.5mM $MgCl_2$, 10mM KCL, 1mM DTT and Protease Inhibitor Cocktail (Sigma–Aldrich, St. Louis, MO). Cellytic TM NuCLEAR^{TM} Extraction Kit (Sigma–Aldrich, St. Louis, MO) was used for isolation of nuclear proteins. Protein concentration in the tissue homogenates was determined by DC protein assay kit (Bio-Rad, U.S.A) and 100 µg of protein per sample was fractionated on 4–12% Novex ®Tris-Glycine gel (Invitrogen, Carlsbad, CA) at 120V for 2h and transferred to nitrocellulose membrane (Invitrogen, Carlsbad, CA). The membrane was incubated for 1h in blocking buffer (1X TBS, 0.05% Tween-20 and 5% nonfat dry milk) and then overnight in the same buffer containing the given primary antibody. Antibodies against Nrf2, nuclear factor-kappa B (NF-$_{\kappa}$B), catalase, glutathione peroxidase (GPX) and Heme Oxygenase-1 (HO-1) were purchased from Sigma-Aldrich Inc (Saint Louis, MO). Mouse monoclonal anti-cyclooxygenase-1, (COX-1) antibody (Millipore, Temecula, CA), mouse polyclonal anti-cyclooxygenase 2 (COX-2) antibody (Cayman Chemical, Ann Arbor, MI), Klotho, nitrotyrosine (Abcam Inc. Cambridge, MA) and sheep polyclonal anti-Cu/Zn superoxide dismutase antibody (Cu/Zn-SOD; Calbiochem Inc. San Diego, CA) were purchased from the cited sources. Antibodies against Histone H1 (Sigma, St. Louis MO) and GAPDH (Sigma, St. Louis MO) were used for measurement of the house keeping nuclear and cytosolic target proteins, respectively. The membrane was washed three times for 10 min in 1X TBST before a 2-h incubation in a buffer (1X TBST) containing horseradish peroxidase-conjugated anti-rabbit (1:3,000) (Abcam), anti-mouse

(1:2000) (GE Healthcare) and anti-sheep (1:5000) IgG (EMD Millipore) secondary antibodies. The membrane was washed three times, then visualized with ECL™ prime western blotting detection reagent (GE Healthcare) and developed by autoluminography. Band densities were quantified using the free Image J software (version 10.2) from the National Institutes of Health (www.imagej.nih.gov/ij/).

2.4 Immunohistochemical Staining

Formalin fixed tissues were paraffin embedded and 7µm sections were placed on glass slides. Following deparaffinization and rehydration in xylene and graded alcohol series, sample were subjected to heat induced antigen retrieval procedure as follows. The slides were placed in citrate buffer (pH 6) and heated in a microwave oven for 2 minutes at high power to reach boiling point, the container was then transferred to a heater to maintain boiling for additional 10 minutes. The samples were then allowed to cool down at room temperature for 30 minutes. After 5 minutes washing in distilled water and 5 minutes in TBS, non-specific binding sites were blocked with protein block solution (BioGenex, Fremont, CA) for 1 hour. Tissue sections were then incubated overnight at 4ºC with primary antibody (Rabbit anti-Nrf2, 1/100, Sigma Aldrich and Rabbit anti-klotho, 1/250, Abcam). The sections were then washed for 3-5 minutes with tris-buffered solution and endogenous peroxidase activity blocked by 3% H_2O_2, followed by a 30-minutes incubation with polymerized anti-rabbit IgG (ImmPRESS, Burlingame, CA). Slides were then rewashed in tris-buffered solution and incubated with peroxidase substrate (DAB ImmPACT, Burlingame, CA) for 2-3 minutes. Sections were counter-stained with Meyer's haematoxylin for 60 seconds, rinsed with tap water, dehydrated in graded alcohol series, cleared in clearing solution and then covered with permount cover slips. Sections were photographed using a Nikon (CLIPSE 80i) light microscope.

2.5 Statistical Analysis

Data are presented as means ± SDs. The non-parametric Mann-Whitney U test was used. P values of less than 0.05 were considered significant. All statistical calculations were performed using SPSS, version 16.0 (SPSS Inc, Chicago, IL).

3. RESULTS

3.1 General Data

Data are summarized in Table 1. Plasma creatinine, urea nitrogen and MDA concentrations, urine protein excretion and arterial pressure were significantly elevated whereas creatinine clearance and hematocrit were significantly reduced in the untreated CKD rats when compared with the control group. Long term administration of dh404 significantly attenuated hypertension and anemia and lowered plasma MDA level. This was associated with partial reductions of plasma creatinine and urea nitrogen concentrations and partial improvement in creatinine clearance.

Table 1. General data in sham-operated control (CTL, n=6), untreated CKD (n=7) and dh404 treated CKD (CKD-Rx, n=8)) groups

	CTL	CKD	CKD-Rx
Body weight (g)	412±4.6	383±14.8	388±12.5
Mean blood pressure (mmHg) at 6 weeks	111±5.5	144±1.6*	117±2.6#
Hematocrit (%)	43.2±0.8	39.9±0.9*	42.8±0.5#
Plasma creatinine (mg/dl)	0.45±0.1	1.35±0.18*	0.81±0.07*#
Plasma urea (mg/dl)	20.4±1.5	82.0±15.2*	38.4±3.1*#
CCr(ml/min/kg)	5.5±3.0	1.85±0.2*	2.7±0.3*
Urine volume (ml/day)	10±0.7	20.0±5.5*	21.0±9.0*
Urine protein (mg/day)	9±5.3	73±8.0*	71.2±4.6*
Plasma malondialdehyde (µmol/l)	0.70±0.13	1.33±0.05*	0.87±0.08#

*Data are mean ± SD: *p<0.05 compared to CTL, #p<0.05 compared to CKD.*

3.2 Renal Histology

The untreated CKD group exhibited significant glomerulosclerosis, tubule-interstitial fibrosis and mononuclear cell infiltrations in the remnant kidney. Administration of Nrf2 activator resulted in partial attenuation glomerulosclerosis, tubulointerstitial fibrosis and inflammation (Fig. 1).

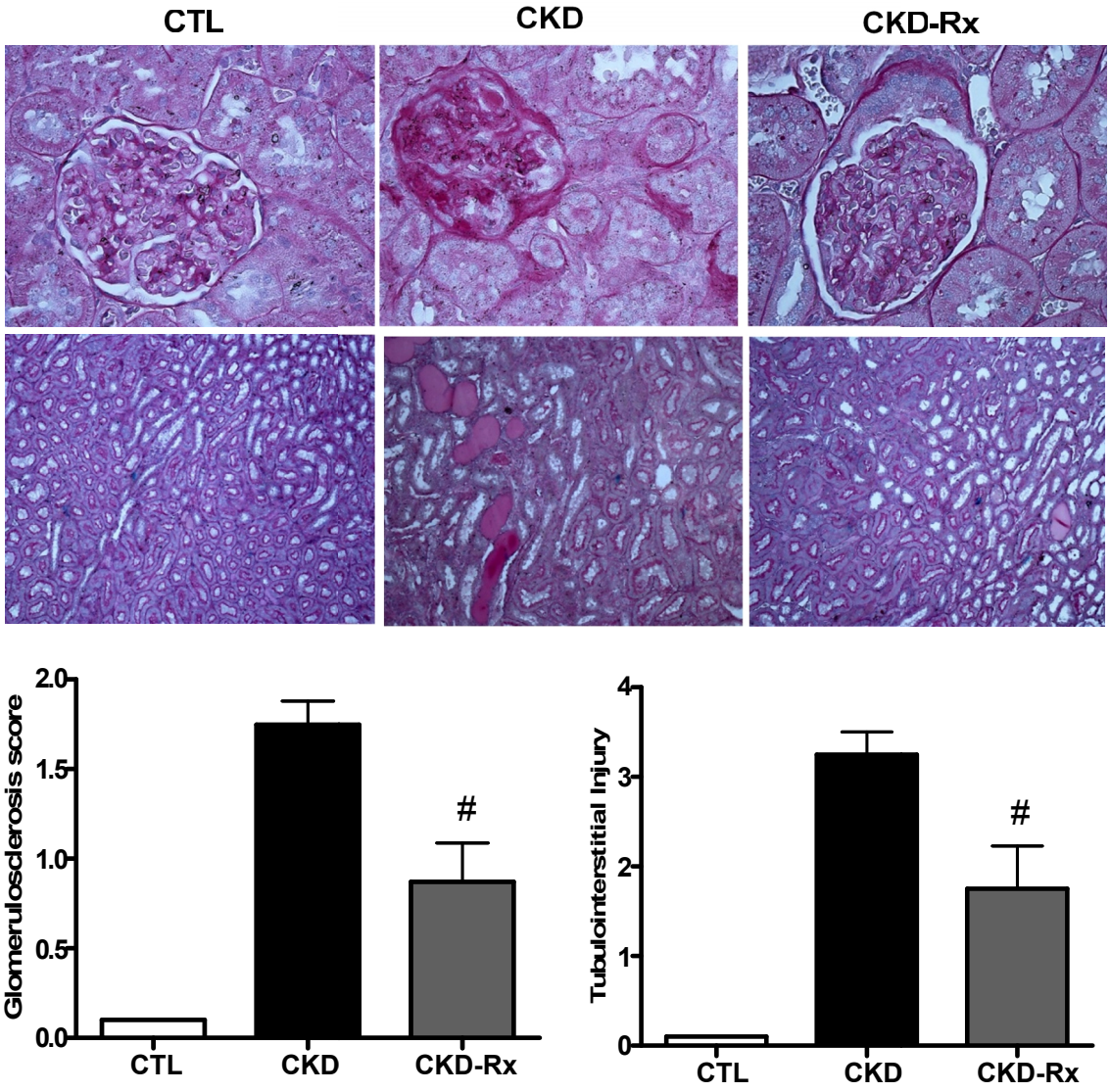

Fig. 1. Representative photomicrographs of the PAS-stained kidney sections depicting glomeruli (Upper panel) and tubulointerstitial (lower panel) region of a normal control (CTL), an untreated CKD and an hd404-treated CKD (CKD-Rx) rat. #p<0.05 compared to CKD

3.3 Nrf2 and Klotho Data

Nuclear Nrf2 content in the untreated CKD group was significantly less than that found in the control group. Administration of dh404 restored nuclear Nrf2 level in the treated CKD rats (Fig. 2). Likewise klotho abundance in the kidney tissue was significantly reduced in the untreated CKD animals and was restored by long-term Nrf2 activator therapy (Fig. 2). The depletion of klotho and defective Nrf2 activity in the untreated CKD group and their restoration in Nrf2 activator-treated group found by Western blot analysis was confirmed by immunohistochemical examinations (Fig.3).

3.4 Oxidative and Inflammatory Pathways

Data are shown in Figs. 4, 5 and 6. Compared with the control group, the untreated CKD group showed significant down-regulation of catalase, Cu/Zn-SOD, GPX and HO-1, activation of NF-κB, up-regulation of COX-1 and COX-2 and accumulation of nitrotyrosine in the remnant kidney. These abnormalities were partially attenuated by Nrf2 activator treatment.

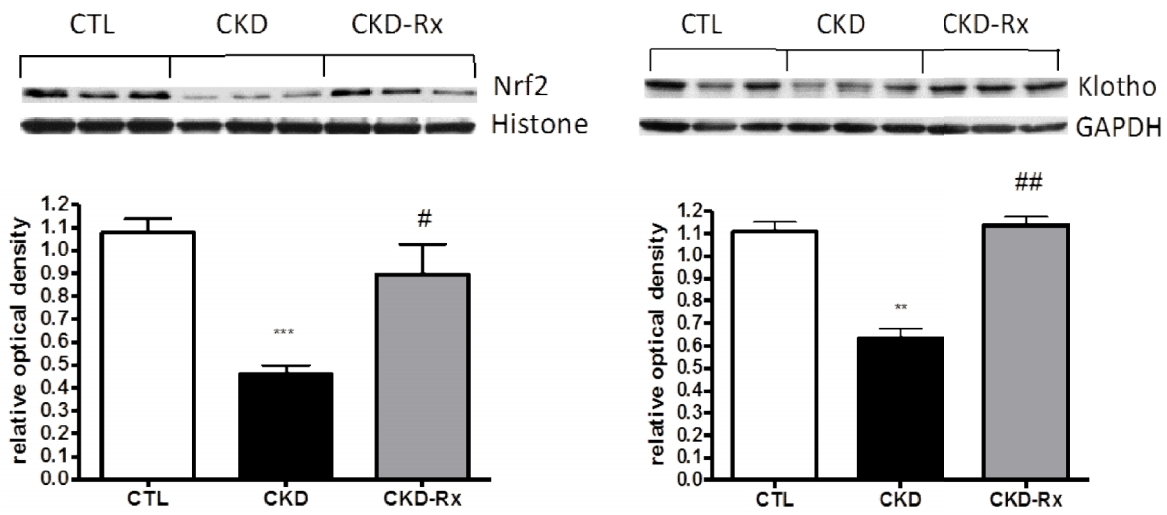

Fig. 2. Representative western blots and bar graphs depicting nuclear Nrf2 content and tissue Klotho expression in the kidneys of the normal control (CTL), untreated CKD and hd404-treated CKD (CKD-Rx) groups **p<0.01 compared to CTL, ***p<0.001 compared to CTL, #p<0.05 compared to CKD, ##p<0.01 compared to CKD

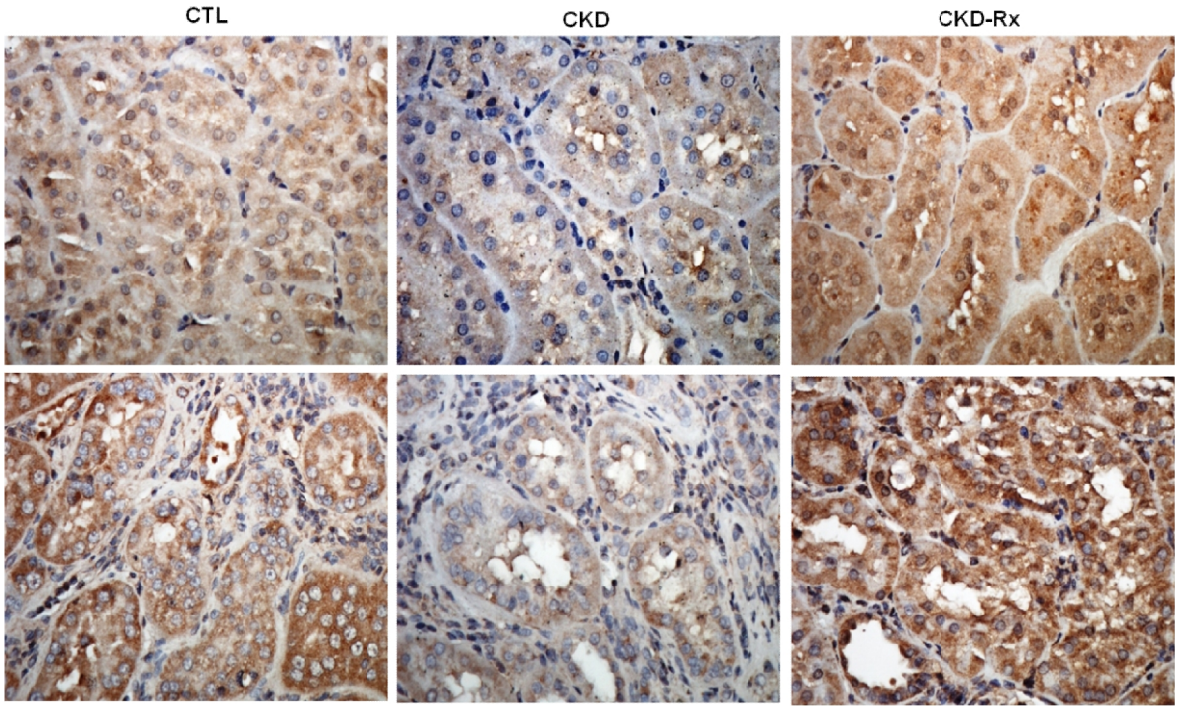

Fig. 3. Representative photomicrography of immunohistochemical staining for klotho (upper) and Nrf2 (lower) of the kidney of a normal control (CTL), an untreated CKD and an hd404-treated CKD (CKD-Rx) rat

4. DISCUSSION

Animals with CKD induced by 5/6 nephrectomy exhibit oxidative stress and inflammation which is due to activation/upregulation of inflammatory cascade, upregulation of ROS producing enzymes and impairment of the endogenous antioxidant defense system [10,18,20-23]. In confirmation of earlier studies [20-22] kidney tissue in our untreated CKD rats exhibited marked reduction of nuclear Nrf2 content which was restored with long-term treatment with

dh404 [29]. These findings confirm the impairment of Nrf2 activity in the diseased kidney and its improvement with dh404. The reduction of Nrf2 activity was accompanied by a parallel reduction in klotho expression in the remnant kidneys of the untreated CKD rats. Administration of dh404 resulted in restoration of renal tissue klotho abundance in the treated CKD group. By limiting the constitutive expression of the antioxidant enzymes and related proteins, impairment of Nrf2 activation in CKD plays a central role in the pathogenesis of the associated oxidative stress and inflammation [24]. This supposition was confirmed by earlier studies which demonstrated the restoration of the key antioxidant enzymes and attenuation of oxidative stress and inflammation in response to long-term treatment with dh404 at the dosage employed in the present study [23]. In confirmation of the latter studies dh404 therapy restored expression of the measured antioxidant enzymes, attenuated NF-κB activity, reversed upregulation of COX-1 and COX-2 expression and lowered interstitial immune cell infiltration.

Attenuation of oxidative stress and inflammation with restoration of Nrf2 activity in our dh404-treated CKD animals was accompanied by reversal of klotho deficiency. Recent studies have demonstrated the association of klotho deficiency with oxidative stress and mitochondrial dysfunction in the kidney and its restoration by attenuation of oxidative stress using N-acetylcystein or AT 1 receptor blockade in animals with cyclosporine nephropathy [14,15). In addition in vitro studies have demonstrated suppression of klotho expression by oxidative stress and its restoration by antioxidants in cultured renal tubular epithelial cells [13].

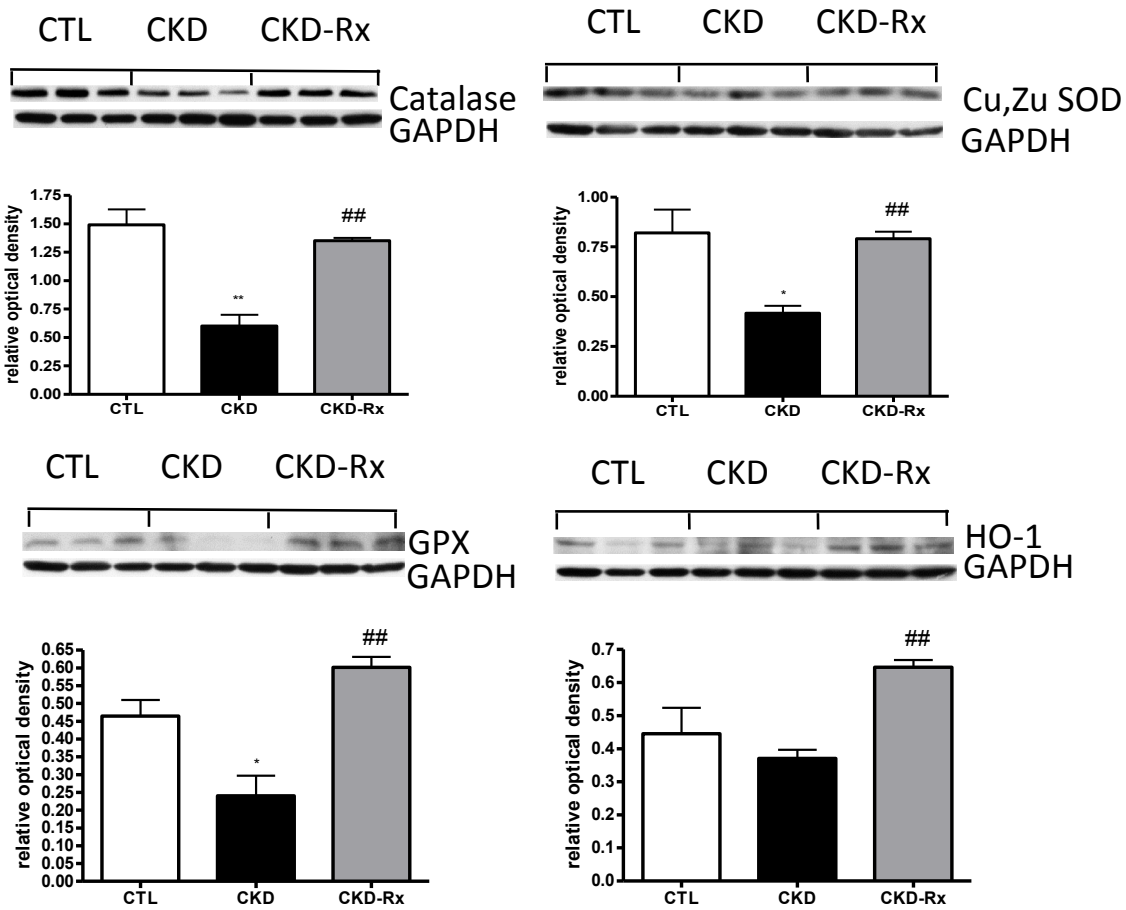

Fig. 4. Representative Western blot and group data depicting relative abundance of catalase, Cu Zn SOD, glutathione peroxidase (GPX) and heme oxigenase-1 (HO-1) in the kidneys of the normal control (CTL), untreated CKD and hd404-treated CKD (CKD-Rx) groups. *p<0.05 compared to CTL, **p<0.01 compared to CTL, ##p<0.01 compared to CKD

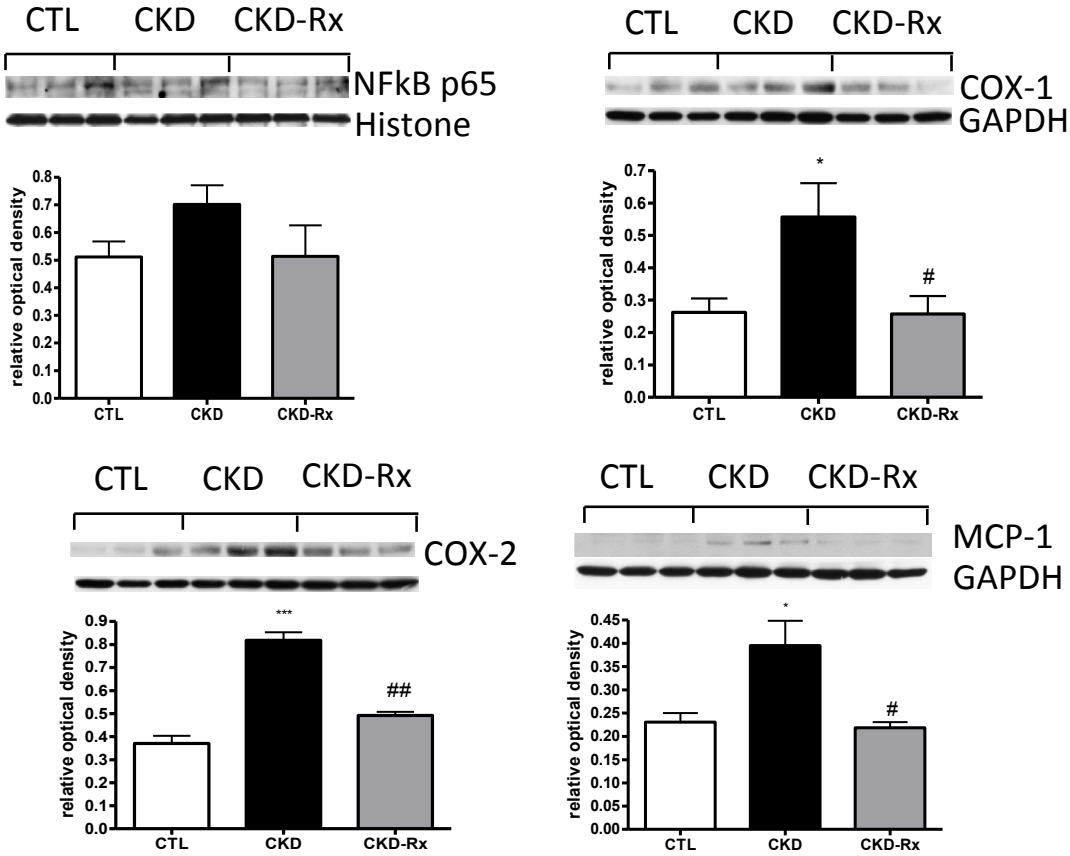

Fig. 5. Representative Western blots and group data depicting nuclear content of P65 subunit of NFkB and tissue abundance of cycloxygenase (COX)-1, cycloxygenase (COX)-2 and MCP-1 in the kidneys of the normal control (CTL), untreated CKD, and hd404-treated CKD (CKD-Rx) groups. *$p < 0.05$ compared to CTL, ***$p < 0.001$ compared to CTL, #$p < 0.05$ compared to CKD, ##$p < 0.01$ compared to CKD

Together these observations support the causal role of oxidative stress in promoting klotho deficiency. This relationship is confirmed by association of klotho deficiency with oxidative stress and its correction with attenuation of oxidative stress in our dh404- treated CKD rats. However a direct effect of the treatment on klotho expression cannot be ruled out and requires further exploration.

Oxidative stress is well known to play a major role in the pathogenesis of hypertension of diverse etiologies [40,41] including CKD-associated hypertension [42]. In fact interventions aimed at attenuating oxidative stress have been shown to improve hypertension in experimental animals [43,44,45]. In confirmation of earlier studies [42], plasma MDA and kidney tissue nitrotyrosine were elevated denoting presence of systemic and local oxidative stress in the untreated CKD group. Restoration of Nrf2 activity and expression of the antioxidant enzymes with dh404 resulted in attenuation of oxidative stress and reduction of ROS-mediated inactivation of NO (as evidenced by reduction of tissue nitrotyrosine abundance), events that can contribute to attenuation of hypertension. Since oxidative stress is well known to promote klotho deficiency, it is reasonable to assume that amelioration of oxidative stress with restoration of Nrf2 activity in the treated CKD group was at least, in part, responsible for the reversal of Klotho deficiency in the study animals. Thus the attenuation of HTN and restoration of Klotho expression in the treated animals were most likely mediated by amelioration of oxidative stress.

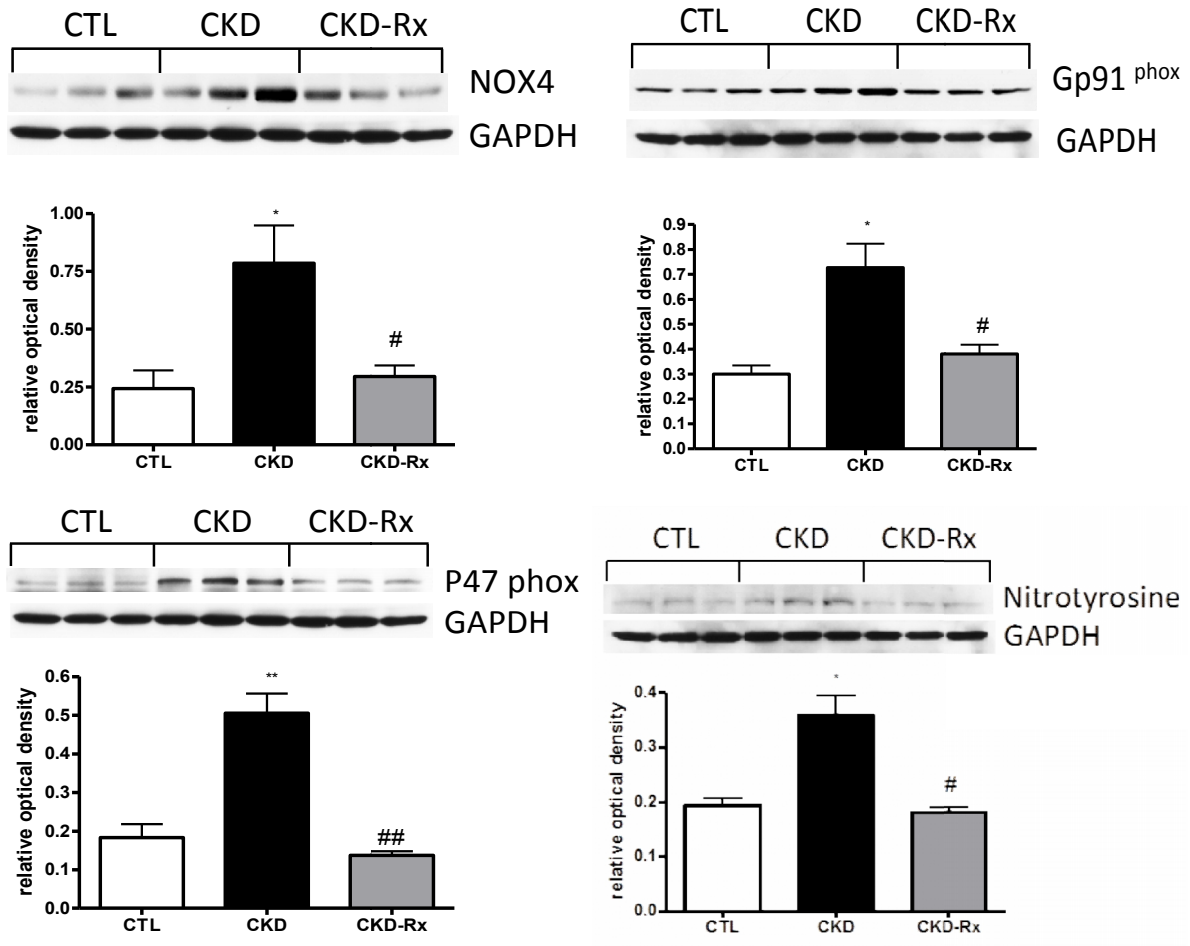

Fig. 6. Representative Western blots and group data depicting NOX4, gp91phox, p47 phox and Nitrotyrosine in the kidneys of the normal control (CTL), untreated CKD and hd404-treated CKD (CKD-Rx) groups. *p<0.05 compared to CTL, **p<0.01 compared to CTL, #p<0.05 compared to CKD, ##p<0.01 compared to CKD

The compound employed in the present study, dh404, belongs to the bardoxolone methyl (CDDO-Methyl ester, BARD) family of synthetic triterpenoid products which have potent Nrf2 activating properties [46,47]. These compounds directly interact with the Nrf2 repressor molecule, keap1, allowing Nrf2 to translocate to the nucleus and enhance expression of its antioxidant and cytoprotective target genes [48]. The phase II-b clinical trial of bardoxolone methyl in patients with advanced CKD and Type 2 diabetes revealed improvement in the estimated glomerular filtration rate. However, this was associated with increased proteinuria, elevated serum transaminase level and higher incidence of adverse events [49]. Moreover due to increased mortality from congestive heart failure the multi-center, randomized, placebo-controlled, Phase 3 trial of bardoxolone methyl in type 2 diabetic patients with stage 4 CKD (BEACON trial) was prematurely terminated [50]. Finally in a recent study Zoja et al [51] found significant weight loss, severe reduction in food intake, elevation in serum transaminase, liver injury and worsening of proteinuria, dyslipidemia, glomerulosclerosis and tubular damage with long term administration of the bardoxolone analogues, RTA 405 (50 and 100 mg/kg/day) and dh404 (5 and 25mg/kg/day) in Zucker rats with type-2 diabetes and nephropathy. Based on these observations the authors questioned the usefulness of the bardoxolone methyl analogs in the management of Type 2 diabetic nephropathy. The reason for the counterintuitive adverse effects of these compounds is presently unknown and awaits further investigation. In an attempt to explore the effect of the drug overdose as a possible cause of the reported adverse effects and to determine the optimal dose of dh404, we conducted a series of preliminary experiments

using a wide range of dh404 dosage between 0.5-20mg/kg/day in male Sprague Dawley rats. We found significant reduction in food intake, heavy weight loss, intense proteinuria and deterioration renal lesions and the gross anatomy of the liver in animals treated with dh404 at doses between 5-20mg/kg. In contrast animals treated with 1-2mg/kg dh404 showed favorable response. Therefore we selected 2mg/kg dosage in the present investigation and two of our other recent studies [23,27].

5. CONCLUSION

In conclusion, administration of Nrf2 activator, dh404, at 2mg/kg/day restored Nrf2 activity, attenuated inflammation, and reversed klotho deficiency in rats with CKD induced by 5/6 nephrectomy. It is unclear whether the favourable effect of therapy with a low dose of this bardoxolone methyl analogue in CKD rats observed here and in earlier studies can be replicated in humans with CKD. Future studies are needed to determine the efficacy of low doses of these compounds in humans with CKD. Future studies are needed to determine the efficacy of low doses of these compounds in humans with CKD.

ACKNOWLEDGENTS

Young Ki Son was supported by research fund from Dong-A University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

1. Wang Y, Sun Z. Current understanding of Klotho. Aging Res Rev. 2009;8:43-51.
2. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI. Mutation of the mouse klotho gene leads to a syndrome resembling aging. Nature. 1997;390:45-51.
3. Doi S, Zou Y, Togao O, Pastor JV, John GB, Shiizaki K, Gotschall R, Schiavi S, Yorioka N, Takahashi M, Boothman DA, Kuro-o M. Klotho inhibits transforming growth factor-beta 1 (TGF-beta1) signaling and suppress renal fibrosis and cancer metastasis in mice. J Biol Chem. 2011;286:8655-8665.
4. Aizawa H, Saito Y, Nakamura T, Inoue M, Imanari T, Ohyama Y, Matsumura Y, Masuda H, Oba S, Mise N, Kimura K, Hasegawa A, Kurabayashi M, Kuro-o M, Nabeshima Y, Nagai R. Downregulation of the klotho gene in the kidney under sustained circulatory stress in rats. Biochem Biophys Res Commun. 1988;249:865-871.
5. Koh N, Fujimori T, Nishiguchi S, Tamori A, Shiomi S, Nakatani T, Sugimura K, Kishimoto T, Kinoshita S, Kuroki T, Nabeshima Y. Severely reduced production of klotho in human chronic renal failure. Biochem Biophysics Res Commun. 2001;280:1015-1020.
6. Hu MC, Shi, M, Zhang J, Quiñones H, Griffith C, Kuro-o M, Moe OW. Klotho deficiency causes vascular calcification in chronic kidney disease. J Am Soc Nephrol. 2011;22:124-136.
7. Haruna Y, Kashihara N, Satoh M, Tomita N, Namokoshi T, Sasaki T, Fujimori T, Xie P, Kanwar YS. Amelioration of progressive renal injury by genetic manipulation of Klotho gene. Proc Natl Acad Sci USA. 2007;104:2331-2336.
8. Hu MC, Shi M, Zhang J, Quiñones H, Kuro-o M, Moe OW. Klotho deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective. Kidney Int. 2010;78:1240-1251.
9. Fujihara CK, Antunes GR, Mattar AL, Malheiros DM, Vieira JM Jr, Zatz R. Chronic inhibition of nuclear factor-kappa B attenuates renal injury in the 5/6 renal ablation model. Am J Physiol Renal Physiol. 2007;292: F92-F99.
10. Vaziri ND, Bai Y, Ni Z, Quiroz Y, Rodriguez-Iturbe B. Intra-renal angiotensin II/AT1 receptor, oxidative stress, inflammation and progressive injury in renal mass reduction. J Pharmacol Exp Ther. 2007;323:85-93.
11. Vaziri ND, Ding Y, Ni Z, Gonick HC. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: Effect of lazaroid therapy. Kidney Int. 1997;52:1042–1046.
12. Manning RD Jr, Tian N, Meng S. Oxidative stress and antioxidant treatment in hypertension and associated renal damage. Am J Nephrol. 2005;25:311-317.
13. Mitobe M, Yoshida T, Sugiura H, Shirota S, Tsuchiya K, Nihei H. Oxidative stress

decreases klotho expression in a mouse kidney cell line. Nephron Exp Nephrol. 2005;101:67-74.
14. Yoon HE, Ghee JY, Piao S, Song JH, Han DH, Kim S, Ohashi N, Kobori H, Kuro-o M, Yang CW. Angiotensin II blockade upregulates the expression of Klotho, the anti-aging gene, in an experimental model of chronic cyclosporine nephropathy. Nephrol Dial Transplant. 2011;26:800-813.
15. Piao SG, Kang SH, Lim SW, Chung BH, Doh KC, Heo SB, Jin L, Li C, Yang CW. Influence of N-acetylcystein on Klotho expression and its signaling pathway in experimental model of chronic cyclosporine nephropathy in mice. Transplantation. 2013;96:146-153.
16. Himmelfarb J, Hakim RM. Oxidative stress in uremia. Curr Opin Nephrol Hypertens. 2003;12:593-598.
17. Vaziri ND, Dicus M, Ho ND, Boroujerdi-Rad L, Sindhu RK. Oxidative stress and dysregulation of superoxide dismutase and NADPH oxidase in renal insufficiency. Kidney Int. 2003;63:179-185.
18. Vaziri ND, Oveisi F, Ding Y. Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. Kidney Int. 1998;53:1748-1754.
19. Yoon JW, Pahl MV, Vaziri ND. Spontaneous leukocyte activation and oxygen-free radical generation in end stage renal disease. Kidney Int. 2007;71:167-172.
20. Kim HJ, Vaziri ND. Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure. Am Physiol Renal Physiol. 2010;298:F662-F671.
21. Kim HJ, Sato T, Rodriguez-Iturbe B, Vaziri ND. Role of intrarenal angiotensin system activation, oxidative stress, inflammation and impaired nuclear factor-erythroid-2-related factor 2 activity in the progression of focal glomerulosclerosis. J Pharmacol Exp Ther. 2011;337:583-590,
22. Aminzadeh MA, Nicholas SB, Norris KC, Vaziri ND. Role of impaired Nrf2 activation in the pathogenesis of oxidative stress and inflammation in chronic tubule-interstitial nephropathy. Nephrol Dial Transplant. 2013;28:2038-2045.
23. Aminzadeh MA, Reisman SA, Vaziri ND, Shelkovnikov S, Farzaneh SH, Khazaeli M, Meyer CJ. The synthetic triterpenoid RTA dh404 (CDDO-dh TFEA) restores endothelial function impaired by reduced Nrf2 activity in chronic kidney disease. Redox Biol. 2013;31:527-531.
24. Ruiz S, Pergola PE, Zager RA, Vaziri ND. Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in Chronic Kidney Disease. Kidney Int. 2013;83:1029-1041.
25. Wakabayashi N, Slocum SL, Skoko JJ, Shin S, Kensler TW. When Nrf2 talks, who's listening? Antioxid Redox Signal. 2010;13:1649-1663.
26. Li W, Khor TO, Xu C, Shen G, Jeong WS, Yu S, Kong AN. Activation of Nrf2-antioxidant signaling attenuates NF-kappaB-inflammatiory response and elicits apoptosis. Biochem Pharmacol. 2008;76:1485-1489.
27. Aminzadeh MA, Reisman SA, Vaziri ND, Khazaeli M, Yuan J, Meyer CJ. The Synthetic Triterpenoid RTA dh404 (CDDO-dh TFEA) restores Nrf2 activity and attenuates oxidative stress, inflammation and fibrosis in rats with chronic renal failure. Xenobiotica; 2013. [Epub ahead of print] PMID: 24195589.
28. Wu QQ, Wang Y, Senitko M, Meyer C, Wigley WC, Ferguson DA, Grossman E, Chen J, Zhou XJ, Hartono J, Winterberg P, Chen B, Agarwal A, Lu CY. Bardoxolone Methyl (BARD) ameliorates ischemic AKI and increases expression of protective genes-Nrf2, PPARγ and HO-1. Am J Physiol Renal Physiol. 2011;300:F1180-F1192.
29. Aleksunes LM, Goedken MJ, Rockwell CE, Thomale J, Manautou JE, Klaassen CD. Transcriptional regulation of renal cytoprotective genes by Nrf2 and its potential use as a therapeutic target to mitigate cisplatin-induced nephrotoxicity. J Pharmacol Exp Ther. 2010;335:2-12.
30. Liu M, Grigoryev DN, Crow MT, Haas M, Yamamoto M, Reddy SP, Rabb H. Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice. Kidney Int. 2009;76:277-285.
31. Zheng H, Whitman SA, Wu W, Wondrak GT, Wong PK, Fang D, Zhang DD. Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. Diabetes. 2011;60:3055-3066.
32. Tsai PY, Ka SM, Chang JM, Chen HC, Shui HA, Li CY, Hua KF, Chang WL, Huang JJ, Yang SS, Chen A. Epigallocatechin-3-gallate prevents lupus nephritis development in mice via

enhancing the N rf2 antioxidant pathway and inhibiting NLRP3 inflammasome activation. Free Radic Biol Med. 2011;51:744-754.
33. Peng A, Ye T, Rakheja D, Tu Y, Wang T, Du Y, Zhou JK, Vaziri ND, Hu Z, Mohan C, Zhou XJ. The green tea polyphenol (-)-epigallocatechin-3-gallate ameliorates experimental immune-mediated glomerulonephritis. Kidney Int. 2011;80:601-611.
34. Sahin K, Tuzcu M, Gencoglu H, Dogukan A, Timurkan M, Sahin N, Aslan A, Kucuk O. Epigallocatechin-3-gallate activates Nrf2/HO-1 signaling pathway in cisplatin-induced nephrotoxicity in rats. Life Sci. 2010;87:240-245.
35. Molina-Jijon E, Tapia E, Zazueta C, El HM, Zatarain-Barron ZL, Hernández-Pando R, Medina-Campos ON, Zarco-Márquez G, Torres I, Pedraza-Chaverri J. Curcumin prevents Cr (VI)-induced renal oxidant damage by a mitochondrial pathway. Free Radic Biol Med. 2011;51:1543-1557.
36. Chen XL, Dodd G, Thomas S, Zhang X, Wasserman MA, Rovin BH, Kunsch C. Activation of Nrf2/ARE pathway protects endothelial cells from oxidant injury and inhibits inflammatory gene expression. Am J Physiol Heart Circ Physiol. 2006;290:H1862-H1870.
37. Dinkova-Kostova AT, Liby KT, Stephenson KK, Holtzclaw WD, Gao X, Suh N, Williams C, Risingsong R, Honda T, Gribble GW, Sporn MB, Talalay P. Extremely potent triterpenoid inducers of the phase 2 response: Correlations of protection against oxidant and inflammatory stress. Proc Natl Acad Sci USA. 2005;102:4584-4589.
38. Honda T, Rounds BV, Bore L, Finlay HJ, Favaloro FG Jr, Suh N, Wang Y, Sporn MB, Gribble GW. Synthetic Oleanane and ursane triterpenoids with modified rings A and C: A series of highly active inhibitors of nitric oxide production in mouse macrophages. J Med Chem. 2000;43:4223-4246.
39. Cho KH, Kim HJ, Rodriguez-Iturbe B, Vaziri ND. Niacin ameliorates oxidative stress, inflammation, proteinuria and hypertension in rats with chronic renal failure. Am J Physiol Renal Physiol. 2009;297:F106–F113.
40. Vaziri ND, Rodriguez-Itrube B. Mechanisms of Disease: Oxidative stress and inflammation in the patogenesis of hypertension. Nature, Reviews Nephrol. 2006;2:582-593.
41. Wilcox CS. Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? Am J Physiol Regul Integr Comp Physiol. 2005;289:R913-935.
42. Vaziri ND, Ni Z, Oveisi F, Liang K. Enhanced nitric oxide inactivation and protein nitration by reactive oxygen species in renal insufficiency. Hypertension. 2002;39:135-141.
43. Schnackenberg CG, Welch WJ, Wilcox CS. Normalization of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic: Role of nitric oxide. Hypertension. 1998;32:59–64.
44. Rodriguez-Iturbe B, Zhan C, Quiroz Y, Sindhu RK, Vaziri ND. Antioxidant-rich diet relieves hypertension and reduces renal immune infiltration in spontaneously hypertensive rats. Hypertension. 2003;41:341-346.
45. Wilcox CS, Pearlman A. Chemistry and antihypertensive effects of tempol and other nitroxides. Pharmacol Rev. 2008;60:418-469.
46. Liby K, Hock T, Yore MM, Suh N, Place AE, Risingsong R, Williams CR, Royce DB, Honda T, Honda Y, Gribble GW, Hill-Kapturczak N, Agarwal A, Sporn MB. The synthetic triterpenoids, CDDO and CDDO-imidazolide, are potent inducers of heme oxygenase-1 and Nrf2/ARE signaling. Cancer Res. 2005;65:4789-4798.
47. Sporn MB, Liby KT, Yore MM, Fu L, Lopchuk JM, Gribble GW. New synthetic triterpenoids: Potent agents for prevention and treatment of tissue injury caused by inflammation and oxidative stress. J Nat Prod. 2011;74:537-545.
48. Yates MS, Tauch M, Katsuoka F, Flanders KC, Liby KT, Honda T, Gribble GW, Johnson DA, Johnson JA, Burton NC, Guilarte TR, Yamamoto M, Sporn MB, Kensler TW. Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes. Mol Cancer Ther. 2007;6:154-162.
49. Pergola PE, Raskin P, Toto RD, Meyer CJ, Huff JW, Grossman EB, Krauth M, Ruiz S, Audhya P, Christ-Schmidt H, Wittes J, Warnock DG. BEAM Study Investigators. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. N Engl J Med. 2011;365:327–336.

50. De Zeeuw D, Akizawa T, Audhya P, Bakris GL, Chin M, Christ-Schmidt H, Goldsberry A, Houser M, Krauth M, Lambers Heerspink HJ, McMurray JJ, Meyer CJ, Parving HH, Remuzzi G, Toto RD, Vaziri ND, Wanner C, Wittes J, Wrolstad D, Chertow GM. BEACON Trial Investigators. Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. N Engl J Med. 2013;369:2492-2503.

51. Zoja C, Corna D, Nava V, Locatelli M, Abbate M, Gaspari F, Carrara F, Sangalli F, Remuzzi G, Benigni A. Analogs of bardoxolone methyl worsen diabetic nephropathy in rats with additional adverse effects. Am J Physiol Renal Physiol. 2013;304:F808–F81.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=683&id=40&aid=6850