HHS Public Access

Author manuscript

Nitric Oxide. Author manuscript; available in PMC 2019 September 29.

Nitrolipids in Kidney Physiology and Disease

Soma Jobbagy[1] and **Roderick J. Tan**[2]

[1]Department of Phamacology, University of Pittsburgh School of Medicine, Pittsburgh, PA

[2]Division of Renal-Electrolyte, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract

The kidneys are vital organs responsible for maintaining body fluid homeostasis within proper physiologic ranges. Kidney disease is an epidemic clinical problem causing significant morbidity and mortality, and current treatments are limited to renin-angiotensin system blockade or renal replacement therapy for the majority of affected individuals. There is a critical, unmet need for novel pharmacological agents to improve the outcome of patients with kidney disease. Nitro-oleic acid (NO_2-OA) is an endogenously generated electrophilic compound with the capacity to modify thiols in proteins, altering their function. The most important targets appear to be the Keap1/Nrf2 and NF-κB pathways, which have widespread effects on antioxidant, detoxifying, and inflammatory responses in cells and tissues. Through these and potentially additional protective actions, NO_2-OA may be capable of preserving or enhancing kidney function in acute and chronic kidney diseases.

Keywords

Acute kidney injury; chronic kidney disease; nitro-oleic acid; inflammation; redox imbalance

Introduction

The kidneys maintain body fluid composition within narrow physiologic ranges, preserving appropriate osmolarity, ionic composition, pH, and volume while excreting metabolic and environmental wastes and toxins. This complex task is achieved by the approximately one million nephrons in each kidney. Each nephron is comprised of a glomerulus performing plasma filtration, and a complex and convoluted renal tubule that refines the composition of what will ultimately become the urine. If these structures become damaged, the kidney is unable to function appropriately.

To whom correspondence should be addressed: Roderick J. Tan, M.D., Ph.D., Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh, 828A Scaife Hall, 3550 Terrace St., Pittsburgh, PA 15261. Phone: (412) 624-4008. Fax: (412) 647-6222. tanrj@upmc.edu.

Nitrated fatty acids (NO_2-FA) are a family of endogenously-produced bioactive electrophiles formed by the nitration of dietary lipids, including linoleic and oleic acids. Nitro-oleic acid (NO_2-OA) is a promising therapeutic NO_2-FA which post-translationally modifies protein thiols through reversible Michael addition, exerting potent effects in diverse disease models.[1–6] Fatty acids in plasma bind to proteins such as albumin and fatty-acid binding proteins through hydrophobic and van der Waals interactions, and as such are not filtered at the glomerulus and are excreted only in minute quantities in the urine. By contrast, electrophilic NO_2-FA are capable of forming reversible covalent Michael adducts with low-molecular weight thiols such as glutathione (GSH), yielding compounds that are significantly more hydrophilic than the parent fatty acid. Following a single oral dose of [^{14}C] 10-NO_2-OA administered to rats, 34.9% of recovered radioactivity was observed in the urine, while 47.9% was present in feces[7]. Both N-acetylcysteine adducts as well as β–oxidation and ω-carboxylation products were detected in the urine, suggesting that GSH adducts are formed and undergo further metabolism. Electrophilic NO_2-FA N-acetylcysteine adducts, cysteine adducts and β–oxidation/ ω-carboxylation metabolites are also detected in the urine of healthy human volunteers[8] and humans receiving an IV formulation of NO_2-OA[9]. It is not definitively known whether urinary NO_2-OA, its conjugates, and its metabolites are derived primarily from filtration or tubular secretion. However, other oxidized lipid mediators such as leukotrienes and prostaglandins are secreted into the tubular lumen as hydrophilic conjugates following Phase II metabolism[10,11]. These results suggest that NO_2-FA and NO_2-FA adducts may reach target cells in the kidney either from the apical or basolateral sides. It has been postulated that the reversibility of the Michael adduct through thiol β-elimination may establish new equilibria and form bioactive NO_2-OA in the glomerular filtrate and urine, leading to cytoprotective signaling activities in the renal tubules and collecting system, as well as distally in the bladder and urinary tract.

Spectrum and Mechanisms of Kidney Disease

Chronic kidney diseases (CKD) are a result of longstanding conditions such as diabetes or hypertension and generally represent permanent and irreversible injury. On the other hand, acute kidney injury (AKI) is usually incited by a specific event such as ischemia, sepsis, or toxin exposures. The term acute tubular necrosis (ATN) has often been used interchangeably for AKI, since renal tubules are usually the focus of injury. While some AKI is reversible and resolves with tubular regeneration, AKI can also progress to permanent CKD if the injury is severe[12]. Reduction-oxidation (redox) imbalance and inflammation play a role in pathogenesis of both AKI and CKD, and may be ameliorated by nitrated fatty acids via the mechanisms discussed below.

Redox imbalance is the overabundance of oxidizing species due to excessive production or insufficient scavenging by antioxidant mechanisms and plays a major role in kidney disease pathology[13]. Reactive species cause persistent cell and tissue damage through off-target oxidation of proteins, lipids, and nucleic acids, and can activate multiple pro-fibrotic and pro-inflammatory signaling cascades, including Nuclear Factor-κB (NF-κB), Transforming Growth Factor-β (TGF-β), and p38 Mitogen Activated Protein Kinase (MAPK)[14–17]. Oxidant generation has been attributed to increased abundance of NADPH oxidase (Nox) enzymes as well as to mitochondrial dysfunction[13,18].

Inflammation is another unifying mechanism in the initiation and progression of kidney disease[16–18]. A variety of inflammatory cells infiltrate the kidney in AKI and CKD[19–21]. Inflammation correlates with loss of renal function in animals and humans, and interventions that limit inflammation are protective[22–24]. Inflammatory cells contribute to tissue scarring/fibrosis via production of transforming growth factor-β (TGF-β) and other growth factors, which stimulate myofibroblast differentiation and deposition of extracellular matrix[25].

NO_2-OA reduces redox imbalance and inflammation through targeting of the Kelch-like ECH-associated protein 1 (Keap1) / Nuclear factor erythroid 2-related factor 2 (Nrf2) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways, respectively. Nrf2 is a transcription factor regulating expression of over 250 genes under the control of the antioxidant response element (ARE) motif. These targets have roles in phase II conjugation reactions, reduction reactions, multidrug resistance, catabolism of oxidants, and biosynthesis of reductants and small-molecule antioxidants such as glutathione[26,27]. Redox- and electrophile-induced modification of critical cysteine thiolates in the Nrf2 repressor Keap1 leads to increased Nrf2 activity to protect the cell from these stressors. Encouragingly, one recent meta-analysis of genome-wide association studies revealed that alterations in Nrf2 signaling is a central hub connecting the metabolic and inflammatory pathways implicated in CKD[28]. A variety of available pharmacological activators of Nrf2, including NO_2-OA, are electrophiles that inactivate the Keap1 repressor[29] and initiate gene expression programs downstream of Nrf2 which prime tissues to respond to insult. It is worth noting that the responses to Nrf2 activation are specific with regards to cell and tissue type, and overlapping but non-identical responses have been observed with different pharmacologic and genetic models of Nrf2 activation[30–32].

NO_2-OA also has anti-inflammatory properties via electrophilic reaction with key cysteine residues in both the NF-κB p65 subunit and a related protein, inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ)[33–36]. Indeed, LPS-induced pro-inflammatory cytokine secretion and leukocyte recruitment were diminished by NO_2-OA[4,33,37,38]. These anti-inflammatory effects of NO_2-OA could occur through direct NF-κB inhibition, but there is evidence for indirect effects since maintenance of a reducing intracellular environment by Nrf2 would dampen NF-κB activation[39–41]. Crosstalk between Nrf2 and NF-kB also occurs, since the attenuation of NF-κB enhances Nrf2 activity through reduced expression of Keap1[42]. Further, NO_2-OA suppresses the signal transducer and activator of transcription (STAT) signaling pathway, which is proinflammatory[34].

NO_2-OA has potential renoprotective effects through engagement of other pathways. Heat shock proteins (HSPs) are protective chaperones upregulated at times of cellular stress, and are upregulated by NO_2-OA[43,44]. Similarly, NO_2-FA including NO_2-OA and nitro-conjugated linoleic acid (NO_2-cLA) enhance expression of heme oxygenase 1 (HO-1), effects that have been well-documented in vascular endothelium[45–47]. HO-1 prevents heme toxicity through its degradation, while simultaneously generating protective biliverdin/bilirubin, carbon monoxide, and upregulating ferritin[48]. These properties are believed to confer protective effects in kidney disease[49]. Yet another mechanism of cellular protection is through peroxisome proliferator-activated receptors (PPARs), with PPAR-γ being upregulated by NO_2-OA[50,51]. Additional pleiotropic actions are suggested by other chapters

in this volume. Taken together, these studies support the primary concept that NO_2-OA may be therapeutic for renal disease through multiple additive or synergistic pathways. The existing experimental trials testing NO_2-OA in kidney-related diseases are summarized in Table 1 and described in detail below.

Acute kidney injury

NO_2-OA may be useful in treating AKI/ATN. The most common experimental model for AKI is ischemia/reperfusion (I/R) injury. In this model, mice were treated with NO_2-OA starting 50 minutes after the injury. Compared to vehicle-treated counterparts, these mice had improved blood urea nitrogen (BUN) and serum creatinine, which are circulating markers of renal function. Nox subunit expression and proinflammatory cytokines MCP-1, IL-1β, and TNF-α were all reduced in the NO_2-OA treated cohort, suggesting dual mechanisms of protection involving redox imbalance and inflammation[1]. Independently, NO_2-OA protected human tubular epithelial cells *in vitro* from an I/R-like oxygen and glucose deprivation/restoration insult, and this effect was also related to suppression of NADPH oxidases[52,53].

NO_2-OA has similar protective effects in AKI caused by sepsis or nephrotoxins. Septic shock causes AKI through cytokine release, hypoperfusion, and ischemia, and can be induced experimentally through injection of lipopolysaccharide (LPS) endotoxin[54–57]. Continuous infusion of NO_2-OA improved both creatinine and BUN while reducing TNF-α and various chemokines and adhesion molecules. NO_2-OA also improved the hypotension related to LPS-induced cardiovascular dysfunction, which would reduce renal ischemia[54]. In an AKI model induced by the nephrotoxin cisplatin, NO_2-OA was again protective, although interpretation must take into account the enhanced detoxification of cisplatin-induced free radicals[58] that would be expected with activation of antioxidant responses by NO_2-OA[59].

At least part of the protection afforded by NO_2-OA is likely mediated by Nrf2 activation. The electrophilic triterpenoid and Nrf2 activator, CDDO-imidazolide (CDDO-Im), reduces AKI injury, and was associated with downregulation of inflammatory cytokines and upregulation of protective antioxidant genes[60]. Hypomorphic Keap1 mice with constitutively active Nrf2 also fared better than wild-type and Nrf2 knockout mice in I/R injury. The Keap1 hypomorphs were additionally protected from the development of late-stage fibrosis (AKI-to-CKD progression), and this was associated with higher glutathione levels which reduce redox imbalance[61]. Pharmacologic activation of Nrf2 by CDDO-Im given in the first five days after I/R injury also protected against fibrosis and CKD development[62]. This last point is important, since it implies that therapy can be initiated after AKI to protect against long-term sequelae. The protective effect of genetic activation of Nrf2 has been corroborated independently in our laboratory, with Keap1 hypomorphic mice demonstrating a reduction in renal inflammatory markers (TNF-α, RANTES, IL-6, and MCP-1) one day after I/R injury. While there was not a corresponding early improvement in renal function or histologic injury, there was reduction in fibrosis at 10 days, suggesting protection against AKI-to-CKD progression[61].

Other effects may also contribute to protection from AKI. Several studies indicate protection from injury with upregulation of HSPs[63–65] and PPAR-γ[66–68]. In addition, induction of HO-1 would be expected to reduce injury[69–71]. While these protective mechanisms have not yet been definitively linked to NO_2-OA in the kidney, it is tempting to speculate that NO_2-OA induces protection in AKI via these pathways.

Chronic Kidney Disease

NO_2-OA has been tested as a treatment for diabetic nephropathy, which is the leading cause of renal failure in the developed world[72,73]. In murine diabetes caused by leptin receptor mutation, NO_2-OA reduced glomerular injury and the expression of profibrotic TGF-β and extracellular matrix proteins collagen III, fibronectin, and α-smooth muscle actin (α-SMA). Synergism between losartan and NO_2-OA was observed on evaluation of inflammatory markers TNF-α, cyclooxygenase 2, and urinary prostaglandin E2 (PGE2) as well as the oxidative stress marker, thiobarbituric acid-reactive substances (TBARS), and renal NAD(P)H oxidase components[74]. NO_2-OA also downregulates the chemoattractant MCP-1 in LPS-activated macrophages,[33,34] which has been strongly implicated in development of diabetic nephropathy[75].

Nrf2 activity may be responsible for these protective effects, as Nrf2 demonstrated beneficial effects in streptozotocin (STZ) models of diabetic nephropathy. Nrf2 null mice suffered more severe CKD than their wild-type counterparts after STZ[76]. Pharmacologic activation of Nrf2 using the natural electrophiles sulforaphane or cinnamic aldehyde mitigated oxidative damage and suppressed pro-fibrotic cytokines, and this effect was dependent on the presence of Nrf2[77]. These results suggest that endogenous lipid electrophiles such as NO_2-OA may reduce diabetic kidney injury via enhancement of Nrf2 activity.

After diabetes, hypertension is the second leading cause of CKD[73]. To date, NO_2-OA has not been evaluated in hypertension models of CKD. However, NO_2-OA has been found to reduce Angiotensin II-induced hypertension through antagonism of the Angiotensin II type 1 receptor[3]. In another model of Angiotensin II infusion, 10-NO_2-OA protected against hypertension and improved vasorelaxation through inhibition of soluble epoxide hydrolase. In addition, dietary supplementation with conjugated linoleic acid and sodium nitrite, which increase production of endogenous NO_2-cLA, mimics the antihypertensive effects of NO_2-OA[78]. Studies evaluating renal function in these hypertension models still need to be performed. The potential to simultaneously reduce blood pressure and inflammation suggests that NO_2- OA may extend the scope of therapeutic benefit to CKD patients with either hypertensive or diabetic disease.

Specific glomerular diseases may also be treated by NO_2-OA. In a mouse model of focal segmental glomerulosclerosis (FSGS) induced by adriamycin, NO_2-OA improved renal function as evidenced by reduced proteinuria, plasma creatinine and blood urea nitrogen[79]. NO_2-OA also improved glomerular and tubulointerstitial lesions on histology as well as collagen III, fibronectin, and α-SMA. As in other models of disease, markers of inflammation (TNF-α, IL-1β, and MCP-1) and reactive oxygen species production (Nox subunits) were also down-regulated in the treatment group, suggesting multiple pathways of

protection. However, as in the cisplatin AKI studies above, activation of Nrf2-linked Phase II enzymes and oxidoreductases by NO_2-OA may have confounding effects on the renal toxicity of adriamycin. Adriamycin is cytotoxic as a result of two competing mechanisms: DNA intercalation resulting in inhibition of Topoisomerase II by the parent compound, or reduction to a reactive semiquinone which generates damaging reactive oxygen species[80]. While some reports suggest that adriamycin is not a substrate for the Nrf2-regulated oxidoreductase NAD(P)H-quinone oxidoreductase (NQO1)[81], others suggest that NQO1 may bioactivate adriamycin and related quinones to reactive semiquinones[82–84], while some evidence suggests that NQO1 may be important in detoxification of adriamycin[85]. Notably, Nrf2 likely activates additional oxidoreductases in addition to NQO1, with effects on adriamycin metabolism which have not been characterized.

Mice with genetic Nrf2 enhancement have demonstrated protection against nonglomerular CKD in a unilateral ureteral obstruction (UUO) model. Animals with constitutive hypomorphism of the Nrf2 repressor Keap1 displayed reduced renal fibrosis and α-SMA compared to wild-type counterparts after UUO. Nrf2 activity was suppressed by the injury in wild-type mice, but this effect was ameliorated by Keap1 hypomorphism[61]. Furthermore, Nrf2 knockout mice were sensitized to UUO, demonstrating greater tubular injury and apoptosis at early stages and greater late-stage fibrosis[86]. These studies indicate that severe injuries can downregulate Nrf2 activity and that loss of this protective pathway is a mechanism for CKD progression.

As in AKI, NO_2-FA are likely to have advantages beyond Nrf2 enhancement. As an endogenously produced molecule, NO_2-FA are less likely to have significant toxicity. This is particularly important since a clinical trial in diabetics using bardoxolone methyl (CDDO-Me), a triterpenoid Nrf2 enhancer, demonstrated an increase in adverse effects leading to early termination of the trial[87,88]. Some preclinical studies verified deleterious effects with pharmacologic Nrf2 enhancement[89]. In contrast, NO_2-FA have targets beyond Nrf2, including NF-κB, HSP, PPAR-γ, and HO-1, which have all been implicated in CKD development[49,90–93]. These pleiotropic actions uniquely position NO_2-FA to have potent beneficial effects in a variety of pathologies.

Conclusion

Novel therapeutics are critically needed for the treatment of kidney disease. By virtue of its potent ability to improve redox imbalance and reduce inflammation, NO_2-OA may be a promising agent for the treatment of both acute and chronic kidney diseases. Additional investigation is required to determine the safety and efficacy of this agent and how it may be best harnessed to improve the care of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by NIH F30 DK108391 (S.J.) and AHA FTF16990086, NIH P30 DK079307, University Of Pittsburgh Medical Center Competitive Medical Research Fund Award, and University of Pittsburgh Physicians Academic Foundation Award (R.J.T.).

References

1. Liu H, et al. Nitro-oleic acid protects the mouse kidney from ischemia and reperfusion injury. AJP Ren. Physiol. 2008; 295:F942–F949.
2. Rudolph V, et al. Endogenous generation and protective effects of nitro-fatty acids in a murine model of focal cardiac ischaemia and reperfusion. Cardiovasc. Res. 2010; 85:155–166. [PubMed: 19666678]
3. Zhang J, et al. Nitro-oleic acid inhibits angiotensin II-induced hypertension. Circ. Res. 2010; 107:540–8. [PubMed: 20558825]
4. Villacorta L, et al. Electrophilic nitro-fatty acids inhibit vascular inflammation by disrupting LPS dependent TLR4 signalling in lipid rafts. Cardiovasc. Res. 2013; 98:116–24. [PubMed: 23334216]
5. Reddy AT, et al. The nitrated fatty acid 10-nitro-oleate attenuates allergic airway disease. J. Immunol. 2013; 191:2053–63. [PubMed: 23913958]
6. Wang W, Li C, Yang T. Protection of Nitro-Fatty Acid against Kidney Diseases. Am. J. Physiol. - Ren. Physiol. 2015; 310 ajprenal.00321.2015.
7. Salvatore SR, Vitturi DA, Fazzari M, Jorkasky DK, Schopfer FJ. Evaluation of 10- Nitro Oleic Acid Bio-Elimination in Rats and Humans. Sci. Rep. 2017; 7:39900. [PubMed: 28054588]
8. Salvatore SR, et al. Characterization and quantification of endogenous fatty acid nitroalkene metabolites in human urine. J. Lipid Res. 2013; 54:1998–2009. [PubMed: 23620137]
9. Salvatore SR, Vitturi DA, Fazzari M, Jorkasky DK, Schopfer FJ. Evaluation of 10-Nitro Oleic Acid Bio-Elimination in Rats and Humans. Sci. Rep. 2017; 7:39900. [PubMed: 28054588]
10. Hinchman CA, Ballatori N. Glutathione conjugation and conversion to mercapturic acids can occur as an intrahepatic process. J. Toxicol. Environ. Health. 1994; 41:387–409. [PubMed: 8145281]
11. Fu J, et al. Metabolomics profiling of the free and total oxidised lipids in urine by LC-MS/MS: application in patients with rheumatoid arthritis. Anal. Bioanal. Chem. 2016; 408:6307–19. [PubMed: 27405874]
12. Basile DP, et al. Progression after AKI: Understanding Maladaptive Repair Processes to Predict and Identify Therapeutic Treatments. J. Am. Soc. Nephrol. 2016; 27:687–697. [PubMed: 26519085]
13. Sedeek M, Nasrallah R, Touyz RM, Hebert RL. NADPH Oxidases, Reactive Oxygen Species, and the Kidney: Friend and Foe. J. Am. Soc. Nephrol. 2013; 24:1512–1518. [PubMed: 23970124]
14. Azad N, et al. Reactive oxygen species-mediated p38 MAPK regulates carbon nanotube-induced fibrogenic and angiogenic responses. Nanotoxicology. 2013; 7:157–68. [PubMed: 22263913]
15. Choi EK, et al. Inhibition of reactive oxygen species downregulates the MAPK pathway in rat spinal cord after limb ischemia reperfusion injury. Int. J. Surg. 2015; 22:74–78. [PubMed: 26283297]
16. Drigotas M, Affolter A, Mann WJ, Brieger J. Reactive oxygen species activation of MAPK pathway results in VEGF upregulation as an undesired irradiation response. J. Oral Pathol. Med. 2013; 42:612–619. [PubMed: 23480207]
17. Nishiyama A, et al. Possible Contributions of Reactive Oxygen Species and Mitogen-Activated Protein Kinase to Renal Injury in Aldosterone/Salt-Induced Hypertensive Rats. Hypertension. 2004; 43:841–848. [PubMed: 14769808]
18. Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC. Oxidative stress, anti oxidant therapies and chronic kidney disease. Nephrology. 2012; 17:311–321. [PubMed: 22288610]
19. Jang HR, Rabb H. Immune cells in experimental acute kidney injury. Nat Rev Nephrol. 2015; 11:88–101. [PubMed: 25331787]
20. Silverstein DM. Inflammation in chronic kidney disease: role in the progression of renal and cardiovascular disease. Pediatr. Nephrol. 2009; 24:1445–52. [PubMed: 19083024]

21. Imig JD, Ryan MJ. Immune and inflammatory role in renal disease. Compr. Physiol. 2013; 3:957–76. [PubMed: 23720336]
22. Yang N, et al. Local macrophage and myofibroblast proliferation in progressive renal injury in the rat remnant kidney. Nephrol. Dial. Transplant. 1998; 13:1967–1974. [PubMed: 9719149]
23. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G. Biomarkers of inflammation and progression of chronic kidney disease. Kidney Int. 2005; 68:237–45. [PubMed: 15954913]
24. Declèves A-E, Sharma K. Novel targets of antifibrotic and anti-inflammatory treatment in CKD. Nat. Rev. Nephrol. 2014; 10:257–67. [PubMed: 24662433]
25. Borthwick LA, Wynn TA, Fisher AJ. Cytokine mediated tissue fibrosis. Biochim. Biophys. Acta. 2013; 1832:1049–60. [PubMed: 23046809]
26. Ma Q, He X. Molecular basis of electrophilic and oxidative defense: promises and perils of Nrf2. Pharmacol. Rev. 2012; 64:1055–81. [PubMed: 22966037]
27. 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. [PubMed: 23325084]
28. Martini S, et al. Integrative biology identifies shared transcriptional networks in CKD. J. Am. Soc. Nephrol. 2014; 25:2559–72. [PubMed: 24925724]
29. Kansanen E, et al. Electrophilic nitro-fatty acids activate Nrf2 by a Keap1 cysteine 151-independent mechanism. J. Biol. Chem. 2011; 286:14019–14027. [PubMed: 21357422]
30. Yates MS, et al. Genetic versus chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression profiles between Keap1 knockout and triterpenoid-treated mice. Carcinogenesis. 2009; 30:1024–1031. [PubMed: 19386581]
31. Kansanen E, et al. Nrf2-dependent and -independent Responses to Nitro-fatty Acids in Human Endothelial Cells. J. Biol. Chem. 2009; 284:33233–33241. [PubMed: 19808663]
32. Zagorski JW, Maser TP, Liby KT, Rockwell CE. Nrf2-Dependent and -Independent Effects of *tert*-Butylhydroquinone, CDDO-Im, and H_2O_2 in Human Jurkat T Cells as Determined by CRISPR/Cas9 Gene Editing. J. Pharmacol. Exp. Ther. 2017; 361:259–267. [PubMed: 28280124]
33. Cui T, et al. Nitrated fatty acids: Endogenous anti-inflammatory signaling mediators. J. Biol. Chem. 2006; 281:35686–98. [PubMed: 16887803]
34. Ichikawa T, et al. Nitroalkenes Suppress Lipopolysaccharide-Induced Signal Transducer and Activator of Transcription Signaling in Macrophages: A Critical Role of Mitogen-Activated Protein Kinase Phosphatase 1. Endocrinology. 2008; 149:4086–4094. [PubMed: 18467446]
35. Musiek ES, et al. Electrophilic cyclopentenone neuroprostanes are anti-inflammatory mediators formed from the peroxidation of the omega-3 polyunsaturated fatty acid docosahexaenoic acid. J. Biol. Chem. 2008; 283:19927–35. [PubMed: 18490445]
36. Lin Y-C, Huang G-D, Hsieh C-W, Wung B-S. The glutathionylation of p65 modulates NF-κB activity in 15-deoxy-Δ 12,14-prostaglandin J_2-treated endothelial cells. Free Radic. Biol. Med. 2012; 52:1844–53. [PubMed: 22387200]
37. Hwang J, Lee KE, Lim J-Y, Park SI. Nitrated fatty acids prevent TNFalpha-stimulated inflammatory and atherogenic responses in endothelial cells. Biochem. Biophys. Res. Commun. 2009; 387:633–40. [PubMed: 19607809]
38. Reddy AT, et al. Endothelial cell peroxisome proliferator-activated receptor γ reduces endotoxemic pulmonary inflammation and injury. J. Immunol. 2012; 189:5411–20. [PubMed: 23105142]
39. Banning A, Brigelius-Flohé R. NF-κB, Nrf2, and HO-1 Interplay in Redox-Regulated VCAM-1 Expression. Antioxid. Redox Signal. 2005; 7:889–899. [PubMed: 15998244]
40. Ganesh Yerra V, Negi G, Sharma SS, Kumar A. Potential therapeutic effects of the simultaneous targeting of the Nrf2 and NF-κB pathways in diabetic neuropathy. Redox Biol. 2013; 1:394–7. [PubMed: 24024177]
41. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. Biochem. Soc. Trans. 2015; 43:621–6. [PubMed: 26551702]
42. Yu M, et al. Nuclear factor p65 interacts with Keap1 to repress the Nrf2-ARE pathway. Cell. Signal. 2011; 23:883–892. [PubMed: 21262351]

43. Zheng R, et al. Regulation of keratinocyte expression of stress proteins and antioxidants by the electrophilic nitrofatty acids 9- and 10-nitrooleic acid. Free Radic. Biol. Med. 2014; 67:1–9. [PubMed: 24140437]
44. Kansanen E, et al. Nrf2-dependent and -independent Responses to Nitro-fatty Acids in Human Endothelial Cells. J. Biol. Chem. 2009; 284:33233–33241. [PubMed: 19808663]
45. Wright MM, et al. Fatty acid transduction of nitric oxide signaling: nitrolinoleic acid potently activates endothelial heme oxygenase 1 expression. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:4299–4304. [PubMed: 16537525]
46. Wright MM, et al. Human haem oxygenase-1 induction by nitro-linoleic acid is mediated by cAMP, AP-1 and E-box response element interactions. Biochem. J. 2009; 422:353–61. [PubMed: 19534727]
47. Khoo NKH, et al. Activation of vascular endothelial nitric oxide synthase and heme oxygenase-1 expression by electrophilic nitro-fatty acids. Free Radic. Biol. Med. 2010; 48:230–9. [PubMed: 19857569]
48. Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. Trends Immunol. 2003; 24:449–455. [PubMed: 12909459]
49. Lever JM, Boddu R, George JF, Agarwal A. Heme Oxygenase-1 in Kidney Health and Disease. Antioxid. Redox Signal. 2016; 25:165–183. [PubMed: 26906116]
50. Baker PRS, et al. Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands. J. Biol. Chem. 2005; 280:42464–42475. [PubMed: 16227625]
51. Schopfer FJ, et al. Covalent Peroxisome Proliferator-activated Receptor γ Adduction by Nitro fatty Acids. J. Biol. Chem. 2010; 285:12321–12333. [PubMed: 20097754]
52. Nie H, et al. Nitro-Oleic Acid Attenuates OGD/R-Triggered Apoptosis in Renal Tubular Cells via Inhibition of Bax Mitochondrial Translocation in a PPAR-gamma Dependent Manner. Cell. Physiol. Biochem. 2015; 35:1201–1218. [PubMed: 25766531]
53. Nie H, et al. Nitro-oleic acid ameliorates oxygen and glucose deprivation/re-oxygenation triggered oxidative stress in renal tubular cells via activation of Nrf2 and suppression of NADPH oxidase. Free Radic. Res. 2016; 50:1200–1213. [PubMed: 27545328]
54. Wang H, et al. Nitro-oleic acid protects against endotoxin-induced endotoxemia and multiorgan injury in mice. AJP Ren. Physiol. 2010; 298:F754–F762.
55. Cunningham PN, et al. Acute renal failure in endotoxemia is caused by TNF acting directly on TNF receptor-1 in kidney. J. Immunol. 2002; 168:5817–23. [PubMed: 12023385]
56. Schrier RW, Wang W. Acute Renal Failure and Sepsis. N. Engl. J. Med. 2004; 351:159–169. [PubMed: 15247356]
57. Gomez H, et al. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. Shock. 2014; 41:3–11.
58. Matsushima H, Yonemura K, Ohishi K, Hishida A. The role of oxygen free radicals in cisplatin-induced acute renal failure in rats. J. Lab. Clin. Med. 1998; 131:518–26. [PubMed: 9626987]
59. Wang H, et al. Nitrooleic Acid Protects against Cisplatin Nephropathy: Role of COX-2/mPGES-1/PGE 2 Cascade. Mediators Inflamm. 2015; 2015:1–9.
60. Liu M, et al. The Nrf2 triterpenoid activator, CDDO-imidazolide, protects kidneys from ischemia reperfusion injury in mice. Kidney Int. 2014; 85:134–41. [PubMed: 24088953]
61. Tan RJ, et al. Keap1 hypomorphism protects against ischemic and obstructive kidney disease. Sci. Rep. 2016; 6:36185. [PubMed: 27804998]
62. Nezu M, et al. Transcription factor Nrf2 hyperactivation in early-phase renal ischemia reperfusion injury prevents tubular damage progression. Kidney Int. 2017; 91:387–401. [PubMed: 27789056]
63. Barrera-Chimal J, et al. Intra-renal transfection of heat shock protein 90 alpha or beta (Hsp90α or Hsp90β) protects against ischemia/reperfusion injury. Nephrol. Dial. Transplant. 2014; 29:301–12. [PubMed: 24166465]
64. Peng Z-Y, et al. The anti-oxidant effects are not the main mechanism for glutamine's protective effects on acute kidney injury in mice. Eur. J. Pharmacol. 2013; 705:11–9. [PubMed: 23454558]

65. Park SW, Chen SWC, Kim M, D'Agati VD, Lee HT. Human heat shock protein 27-overexpressing mice are protected against acute kidney injury after hepatic ischemia and reperfusion. Am. J. Physiol. Renal Physiol. 2009; 297:F885–94. [PubMed: 19656912]
66. Chen D-Z, et al. Esculentoside A inhibits LPS-induced acute kidney injury by activating PPAR-γ. Microb. Pathog. 2017; 110:208–213. [PubMed: 28666844]
67. Mohey V, et al. Sildenafil obviates ischemia-reperfusion injury-induced acute kidney injury through peroxisome proliferator-activated receptor γ agonism in rats. J. Surg. Res. 2016; 201:69–75. [PubMed: 26850186]
68. Wu QQ, et al. Bardoxolone methyl (BARD) ameliorates ischemic AKI and increases expression of protective genes Nrf2, PPARγ, and HO-1. Am. J. Physiol. Renal Physiol. 2011; 300:F1180–92. [PubMed: 21289052]
69. Nath KA, et al. Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. J. Clin. Invest. 1992; 90:267–70. [PubMed: 1634613]
70. Ferenbach DA, et al. Macrophages expressing heme oxygenase-1 improve renal function in ischemia/reperfusion injury. Mol. Ther. 2010; 18:1706–13. [PubMed: 20551909]
71. Bolisetty S, Traylor A, Joseph R, Zarjou A, Agarwal A. Proximal tubule-targeted heme oxygenase-1 in cisplatin-induced acute kidney injury. Am. J. Physiol. Renal Physiol. 2016; 310:F385–94. [PubMed: 26672618]
72. Johnson SA, Spurney RF. Twenty years after ACEIs and ARBs: emerging treatment strategies for diabetic nephropathy. Am. J. Physiol. Physiol. 2015; 309:F807–F820.
73. Saran R, et al. US Renal Data System 2015 Annual Data Report: Epidemiology of Kidney Disease in the United States. American journal of kidney diseases : the official journal of the. National Kidney Foundation. 2016; 67
74. Liu Y, et al. Combined losartan and nitro-oleic acid remarkably improves diabetic nephropathy in mice. AJP Ren. Physiol. 2013; 305:F1555–F1562.
75. Wada T, et al. Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy. Kidney Int. 2000; 58:1492–9. [PubMed: 11012884]
76. Yoh K, et al. Hyperglycemia induces oxidative and nitrosative stress and increases renal functional impairment in Nrf2-deficient mice. Genes Cells. 2008; 13:1159–1170. [PubMed: 19090810]
77. Zheng H, et al. Therapeutic Potential of Nrf2 Activators in Streptozotocin-Induced Diabetic Nephropathy. Diabetes. 2011; 60:3055–3066. [PubMed: 22025779]
78. Nie H, et al. Nitro-oleic acid attenuates OGD/R-triggered apoptosis in renal tubular cells via inhibition of bax mitochondrial translocation in a ppar-gamma dependent manner. Cell. Physiol. Biochem. 2015; 35:1201–1218. [PubMed: 25766531]
79. Liu S, et al. Nitro-oleic acid protects against adriamycin-induced nephropathy in mice. AJP Ren. Physiol. 2013; 305:F1533–F1541.
80. Thorn CF, et al. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenet. Genomics. 2011; 21:440–6. [PubMed: 21048526]
81. Wallin R. Adriamycin and DT-diaphorase. Cancer Lett. 1986; 30:97–101. [PubMed: 3080227]
82. Sinha BK, Trush MA, Kennedy KA, Mimnaugh EG. Enzymatic Activation and Binding of Adriamycin to Nuclear DMA1. CANCER Res. 1984; 44:2892–2896. [PubMed: 6327028]
83. Ross D, Siegel D. NAD(P)H:Quinone Oxidoreductase 1 (NQO1, DT-Diaphorase), Functions and Pharmacogenetics. Methods Enzymol. 2004; 382:115–144. [PubMed: 15047100]
84. Thorn CF, et al. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenet. Genomics. 2011; 21:440–6. [PubMed: 21048526]
85. Zeekpudsa P, Kukongviriyapan V, Senggunprai L, Sripa B, Prawan A. Suppression of NAD(P)H-quinone oxidoreductase 1 enhanced the susceptibility of cholangiocarcinoma cells to chemotherapeutic agents. J. Exp. Clin. Cancer Res. 2014; 33:11. [PubMed: 24460787]
86. Kong W, et al. Nrf2 deficiency promotes the progression from acute tubular damage to chronic renal fibrosis following unilateral ureteral obstruction. Nephrol. Dial. Transplant. 2017; doi: 10.1093/ndt/gfx299
87. de Zeeuw D, et al. Bardoxolone Methyl in Type 2 Diabetes and Stage 4 Chronic Kidney Disease. N. Engl. J. Med. 2013; 369:2492–2503. [PubMed: 24206459]

88. Chin MP, et al. Risk Factors for Heart Failure in Patients With Type 2 Diabetes Mellitus and Stage 4 Chronic Kidney Disease Treated With Bardoxolone Methyl. J. Card. Fail. 2014; 20:953–958. [PubMed: 25307295]

89. Zoja C, et al. Analogs of bardoxolone methyl worsen diabetic nephropathy in rats with additional adverse effects. Am. J. Physiol. Physiol. 2013; 304:F808–F819.

90. Musiał K, Zwolińska D. Heat shock proteins in chronic kidney disease. Pediatr. Nephrol. 2011; 26:1031–7. [PubMed: 21193931]

91. Lapice E, et al. The PPARγ2 Pro12Ala variant is protective against progression of nephropathy in people with type 2 diabetes. J. Transl. Med. 2015; 13:85. [PubMed: 25889595]

92. Jiang L, et al. The potential signaling pathway between peroxisome proliferator-activated receptor gamma and retinoic acid receptor alpha in renal interstitial fibrosis disease. J. Recept. Signal. Transduct. Res. 2015; 35:258–68. [PubMed: 25359573]

93. Declèves A-E, Sharma K. Novel targets of antifibrotic and anti-inflammatory treatment in CKD. Nat. Rev. Nephrol. 2014; 10:257–67. [PubMed: 24662433]

94. Bonacci G, et al. Conjugated linoleic acid is a preferential substrate for fatty acid nitration. J. Biol. Chem. 2012; 287:44071–44082. [PubMed: 23144452]

Nitric Oxide. Author manuscript; available in PMC 2019 September 29.

Table 1

Summary of literature describing renal effects of NO_2-OA

Relevant information from each publication including species/strain, injury model, drug and dosing, as well as a summary of findings, are included.

Reference	Species	Model	Drug	Dose	Effect of Drug
1	Mouse (B6129SF2/J)	AKI - Bilateral I/R (30min)	NO_2-OA	500μg/kg starting 50min after ischemia and every 6 hours for 24 hours.	↓ histologic injury score, plasma Cre/BUN, and tissue injury markers (MPO, TNF- α, ICAM-1, IL-1β, NOX,)
52	Human (tubular epithelial cell)	AKI – Oxygen/Glucose Deprivation	NO_2-OA	1.25μM for 45min prior to deprivation	↓ Bax activation and inhibition of apoptotic cascade
53	Human (tubular epithelial cell)	AKI – Oxygen/Glucose Deprivation	NO_2-OA	1.25μM for 45min prior to deprivation	↓ NOX2/4, ↑ Nrf2 targets
54	Mouse (C57BL/6J)	AKI – Sepsis (10mg/kg LPS I.P.)	NO_2-OA	200μg/kg*d (osmotic minipump) starting 48hr prior to LPS exposure	↓ Inflammation, cardiac, hepaBc, renal Bssue injury (↓ Renal MCP-1, ICAM-1, VCAM-1, iNOS, COX-2, PGE_2 and ↓ Plasma Cre/BUN)
59	Mouse (C57BL/6)	AKI – Cisplatin (20mg/kg I.P.)	NO_2-OA	2mg/kg*d (given for 48hr as pre-treatment only via osmotic minipump)	↓ Plasma Cre/BUN; ↓ histologic injury score Caveat: NO_2-OA may have indirect effects on cisplatin metabolism leading to lower exposure
74	Mouse (db/db)	CKD - Diabetic nephropathy	NO_2-OA + Losartan	5mg/kg*d (NO_2-OA, osmotic minipump) and 10mg/kg*d (losartan, diet) for 2 weeks	↓ markers of oxidaBve stress (TBARS, NOX2/4) and inflammation (TNF-α, COX-2, urine PGE_2), ↓ glomerulosclerosis and fibrosis
3	Mouse (C57BL/6J)	CKD – Ang II hypertension (500ng/kg*min osmotic minipump)	NO_2-OA GW9622	5mg/kg*d (osmotic minipump) or 10mg/kg (jugular vein infusion); 10mg/kg (jugular vein infusion)	↓ systolic and diastolic arterial pressure Effects were PPAR-γ independent
	Rat (2nd order mesenteric artery)	Ang II vasoconstriction dose response	NO_2-OA	2.5–5μM	↓ mesenteric artery contraction by Ang II
78	Mouse (C57BL/6) sEH C521S mut and WT	CKD – Ang II hypertension (1mg/kg*d osmotic minipump)	NO_2-OA	5mg/kg*d (osmotic minipump) starting 3 days after Ang II exposure	↓Mean arterial pressure, cardiac hypertrophy; inhibition of sEH; ↑ vasorelaxation; effects were negated in sEH “redox dead“ mutant mice
			Conjugated linoleic acid + sodium nitrite (to generate nitro fatty acids)	10mg/kg*d cLA + 20mg/kg*d $NaNO_2$ 5 days oral gavage	Inhibition of sEH Generation of NO_2-FA (Documented with same dietary protocol in independent manuscript[94])

Reference	Species	Model	Drug	Dose	Effect of Drug
[79]	Mouse (BALB/c)	AKI – Adriamycin (10mg/kg I.P.)	NO_2-OA	5mg/kg*d (osmotic minipump) starting 2 days prior to adriamycin exposure	↓ glomerulosclerosis, podocyte loss, tubulointersBBal fibrosis, oxidative stress Caveat: NO_2-OA may have indirect effects on adriamycin metabolism leading to lower exposure

Abbreviations: Angiotensin II (Ang II), Bcl-associated X protein (Bax), blood urea nitrogen (BUN), Creatinine (Cre), Cyclooxygenase 2 (COX-2), PPAR-γ inhibitor (GW9622), inducible Nitric Oxide Synthase (iNOS), Intercellular Adhesion Molecule 1 (ICAM-1), Interleukin 1 Beta (IL-1β), intraperitoneal (I.P.), Lipopolysaccharide (LPS), Monocyte Chemoattractant Protein 1 (MCP-1), Myeloperoxidase (MPO), NAD(P)H Oxidase (NOX), nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2), Prostaglandin E2 (PGE_2), soluble Epoxide Hydrolase (sEH), Tissue Necrosis Factor alpha (TNF-α), Vascular Cell Adhesion Molecule 1 (VCAM-1), Thiobarbituric acid reactive substances (TBARS).