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ORIGINAL ARTICLE

Role of Nrf2 Dysfunction in Uremia-Associated Intestinal Inflammation and Epithelial Barrier Disruption

Wei Ling Lau · Shu-Man Liu · Sogol Pahlevan · Jun Yuan · Mahyar Khazaeli · Zhenmin Ni · Jefferson Y. Chan · Nosratola D. Vaziri

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Background Gut inflammation is prevalent in chronic kidney disease (CKD) and likely contributes to systemic inflammation via disruption of the epithelial tight junction with subsequent endotoxin and bacterial translocation.
Aims To study the expression profile of inflammatory and tight junction proteins in the colon from CKD rats compared to healthy controls, and demonstrate the role of Nrf2 (transcription factor nuclear factor erythroid 2-related factor 2) using a potent Nrf2 activator.
Methods CKD was induced via 5/6 nephrectomy in Sprague–Dawley rats, and dh404 (2 mg/kg/day) was used to study the effects of systemic Nrf2 activation. The experimental groups included sham, CKD and CKD? dh404 rats. Blood and colon tissues were analyzed after a 10-week study period.
Results Colon from CKD rats showed histological evidence of colitis, depletion of epithelial tight junction proteins, significant reduction of Nrf2 and its measured target gene products (NQO1, catalase, and CuZn SOD), activation of NFkB, and upregulation of pro-inflammatory molecules (COX-2, MCP-1, iNOS, and gp91^{phox}). Treatment with dh404 attenuated colonic inflammation, restored Nrf2 activity and levels of NQO1, catalase and CuZn SOD, decreased NFkB and lowered expression of COX-2, MCP-1, iNOS, and gp91^{phox}. This was associated with restora- tion of colonic epithelial tight junction proteins (occludin and claudin-1).
Conclusions CKD rats exhibited colitis, disruption of colonic epithelial tight junction, activation of inflammatory mediators, and impairment of Nrf2 pathway. Treatment with an Nrf2 activator restored Nrf2 activity, attenuated colonic inflammation, and restored epithelial tight junction proteins.

Keywords Colon inflammation Epithelial tight junction Chronic kidney disease Nrf2

Introduction

Impairment of the intestinal barrier (‘‘leaky gut’’) is now increasingly recognized as a pathogenic mechanism in a number of chronic non-infectious diseases including heart failure, nonalcoholic fatty liver disease, diabetes mellitus, and fibromyalgia [1–3]. Almost 30 years ago, Vaziri et al. [4] showed the presence of inflammation throughout the gastrointestinal tract (including gastritis, enteritis, and colitis) in a postmortem analysis of 78 hemodialysis patients. Recent studies by our group have demonstrated disruption of the epithelial tight junction throughout the gastrointestinal tract and its association with endotoxemia, local and systemic inflammation, and oxidative stress in animal models of CKD [5–7]. Subsequent in vitro studies revealed the role of urea and its conversion to ammonia and

Part of this study was presented in form at the American Society of Nephrology Kidney Week 2013 in Atlanta, GA.

Wei Ling Lau and Shu-Man Liu have contributed equally to this work.

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ammonium hydroxide as a main cause of the breakdown of the gut epithelial tight junction [8, 9]. In addition, the intestinal wall inflammation can lead to disruption of the epithelial barrier structure by retraction and endocytosis of the transcellular tight junction proteins, claudins and occludin [10–12]. Disruption of the epithelial barrier structure and function in turn amplifies local and systemic inflammation by allowing translocation of bacterial toxins and other noxious products into the intestinal wall and systemic circulation. Indeed, inflammation in the CKD population is frequently associated with endotoxemia without signs of clinical infection [13, 14], and recent papers highlight bacterial translocation and its association with systemic inflammation in end-stage renal disease (ESRD) patients maintained on hemodialysis [15, 16]. Serum pro-inflammatory cytokine levels (IL-1, TNF-a, IL- 6, IL-13) and circulating endotoxin are significantly asso- ciated with increased mortality in hemodialysis patients [17–19], and systemic inflammation is strongly associated with comorbidities including cardiovascular disease, ane- mia, and malnutrition [20–22].

Intestinal wall inflammation and barrier dysfunction in ESRD patients and CKD rats are accompanied by marked alteration of the gut microbiome [23] and dominance of urease-possessing bacterial families [24]. Heavy influx of urea in the gut lumen together with bacterial urease in humans and animals with CKD facilitates generation of ammonia and ammonium hydroxide [25, 26] and the erosion of the epithelial tight junction and local inflammation [9]. Local inflammation, in turn, amplifies disruption of epithelial tight junction by promoting endocytosis of claudins and occludin as mentioned above.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that plays a central role in the regulation of antioxidant and phase 2 detoxifying enzymes and related proteins [27, 28]. In the cytoplasm, Keap1 (Kelch-like ECH-associated protein 1) binds to Nrf2 and facilitates its ubiquitination and thereby prevents its translocation to the nucleus. Increase in intracellular reactive oxygen species enhances nuclear translocation of Nrf2 and expression of its target genes by modifying the reactive cysteine residues on Keap1, thus limiting its ability to bind Nrf2 [29, 30]. Oxidative stress and inflammation in CKD are associated with marked elevation of Keap1 abundance, impaired Nrf2 activity, and significant down-regulation of Nrf2 target gene products in the kidney and vascular tissues [31, 32]. The renal protective effect of Nrf2 is supported by the fact that Nrf2 knockout mice develop lupus-like auto- immune nephritis [33] and show intensified oxidative stress with accelerated renal injury when subjected to experi- mental diabetes mellitus [34]. Further, Nrf2-deficient mice subjected to ischemic and nephrotoxic insults show impaired upregulation of antioxidant pathways, with more severe acute kidney injury and higher mortality, compared to wild-type controls [35]. Finally, recent studies have demonstrated the salutary effects of a low dose of Nrf2 inducer dh404 on oxidative stress, kidney fibrosis, endothelial dysfunction, and vascular abnormalities in CKD animals [36, 37]. However, to our knowledge, the impact of CKD on the intestinal Nrf2 pathway and the effect of Nrf2 inducers on CKD-associated gut inflammation and barrier disruption have not previously been described.

Given the contribution of gut pathology to systemic inflammation, the central role of Nrf2 in defense against oxidative stress and inflammation, and recent demonstration of renal and vascular Nrf2 deficiency in CKD, we examined the Nrf2 pathway in intestinal tissue and the response to a potent Nrf2 inducer in CKD rats.

Methods

Experimental Groups

Male Sprague–Dawley rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN). They were randomized to undergo sham operation (controls) or 5/6 nephrectomy (CKD) by resection of both poles of the decapsulated left kidney, followed by right total nephrectomy 5 days later. Surgeries were performed under general anesthesia using intraperitoneal injection of ketamine/xylazine. Experimental groups were as follows: sham controls (CTL, $n = 5$), vehicle-treated CKD ($n = 8$), and CKD treated with Nrf2 activator dh404 (CKD ? dh404, $n = 8$). Tail blood pressure was measured at 6 weeks. All experiments were approved by the University of California, Irvine Institutional Committee for the Use and Care of Experimental Animals.

The Nrf2 activator analog synthetic triterpenoid dh404 (2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid-9,11-dihydro-trifluoroethyl amide or CDDO-dhTFEA) was obtained from Reata Pharmaceuticals (Irving, TX). dh404 interrupts Keap1-mediated Nrf2 ubiquitination by saturating the binding capacity of Keap1, thus facilitating increased Nrf2 translocation to the nucleus [38]. The drug was dissolved in sesame oil and administered by daily oral gavage (2 mg/kg/day) for 10 weeks. The given dh404 dosage was selected by conducting a series of preliminary dose response experiments using doses ranging 0.5–20 mg/kg/day. We found significant weight loss, marked increase in proteinuria, and worsening of kidney pathology in animals treated with doses between 5 and 20 mg/kg, but significant improvements were noted at doses of 1–2 mg/kg.

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