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Altered intestinal microbial flora and impaired epithelial barrier structure and function in CKD: the nature, mechanisms, consequences and potential treatment

Nosratola D. Vaziri, Ying-Yong Zhao and Madeleine V. Pahl

Division of Nephrology and Hypertension, Department of Medicine, University of California, Irvine Medical Center, Orange, CA, USA

Correspondence and offprint requests to: Nosratola D. Vaziri; E-mail: ndvaziri@uci.edu

ABSTRACT

Chronic kidney disease (CKD) results in systemic inflammation and oxidative stress which play a central role in CKD progression and its adverse consequences. Although many of the causes and consequences of oxidative stress and inflammation in CKD have been extensively explored, little attention had been paid to the intestine and its microbial flora as a potential source of these problems. Our recent studies have revealed significant disruption of the colonic, ileal, jejunal and gastric epithelial tight junction in different models of CKD in rats. Moreover, the disruption of the epithelial barrier structure and function found in uremic animals was replicated in cultured human colonocytes exposed to uremic human plasma *in vitro*. We have further found significant changes in the composition and function of colonic bacterial flora in humans and animals with advanced CKD. Together, uremia-induced impairment of the intestinal epithelial barrier structure and function and changes in composition of the gut microbiome contribute to the systemic inflammation and uremic toxicity by accommodating the translocation of endotoxin, microbial fragments and other noxious luminal products in the circulation. In addition, colonic bacteria are the main source of several well-known pro-inflammatory uremic toxins such as indoxyl sulfate, *p*-cresol sulfate, trimethylamine-*N*-oxide and many as-yet unidentified retained compounds in end-stage renal disease patients. This review is intended to provide an overview of the effects of CKD on the gut microbiome and intestinal epithelial barrier structure and their role in the pathogenesis of systemic inflammation and uremic toxicity. In addition, potential interventions aimed at mitigating these abnormalities are briefly discussed.

Keywords: cardiovascular disease, end-stage renal disease, microbiome, oxidative stress, uremia

INTRODUCTION

Systemic inflammation is invariably present and plays a major role in the progression of chronic kidney disease (CKD) and the associated complications including cardiovascular disease, cachexia and anemia among others [1–5]. Plasma concentration of pro-inflammatory cytokines and chemokines is consistently elevated and circulating leukocytes are activated and produce reactive oxygen species in end-stage renal disease (ESRD) patients [6–10]. Systemic inflammation is invariably accompanied by oxidative stress in humans and animals with CKD. Oxidative stress and inflammation are inseparably linked as each causes and intensifies the other. By activating the transcription factor, NFκB (the master regulator of pro-inflammatory cytokines and chemokines) and by promoting formation of oxidized lipoproteins and glycoxidation products, oxidative stress provokes inflammation. Conversely inflammation causes oxidative stress by triggering production of reactive oxygen, nitrogen and halogen species by the activated immune cells. Systemic inflammation in CKD and ESRD patients commonly results in the reduction of serum albumin, total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol and transferrin saturation and elevation of serum triglycerides and ferritin levels. The main clinical features and consequences of inflammation in CKD patients include frailty, impaired physical strength, erythropoietin-resistant anemia and increased risk of cardiovascular events, hospitalization and death [11].

Several factors contribute to the pathogenesis of CKD-associated oxidative stress and inflammation including (1) retained uremic toxins and metabolites, (ii) comorbid conditions (e.g. diabetes and autoimmune diseases), (iii) activation of tissue angiotensin system, (iv) mitochondrial dysfunction, (v) hypervolemia and hypertension, (vi) infections (blood access, PD catheters, hepatitis etc.), (vii) impairment of the

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Nrf2 pathway, which is the master regulator of the endogenous antioxidant and cytoprotective molecules [12–15] and (viii) iatrogenic factors such as blood–dialyzer membrane interaction, influx of impurities from the dialysate compartment across the high-flux dialysis membranes and indiscriminate use of intravenous iron preparations [16–18], and finally (ix) profound changes in the composition and function of the intestinal microbiome and disruption of the gut epithelial barrier structure leading to generation and influx of endotoxin, microbial fragments and other toxic and pro-inflammatory products in the body's internal milieu. This article is intended to provide an overview of the CKD-associated changes in the structure and function of the gut microbiome and intestinal epithelial barrier structure.

THE NORMAL INTESTINAL MICROBIAL FLORA AND ITS BIOLOGICAL FUNCTIONS

The gastrointestinal tract is home to a large community of microbes (microbiome) that have a symbiotic relationship with the host. The entire length of the alimentary tract is colonized with bacteria and the density of the bacteria progressively increases from 10^2–10^4/mL in the stomach, 10^6–10^8/mL in the ileum, and to $>10^{12}$ microbes/mL in the colon. The enormity of the gut microbiome is evidenced by the fact that it accounts for over 65% of the weight of the fecal material and that the number of the microorganisms residing in the gut far exceeds the total number of cells in the body [19]. Recent progress in the development of molecular-genetic sequencing techniques via extraction of bacterial DNA and 16S ribosomal RNA has resulted in a more thorough characterization and understanding of the gut microbiome in health and disease. While a healthy microbiome is defined by the diversity in bacterial species that can provide a varied repertoire of microbial metabolic functions [20], there is remarkable commonality among individuals. Over 50% of healthy individuals have the same 75 bacterial species in common and bacteria from only 7–9 phyla (from the 55 known bacterial phyla) are detected in humans. Over 90% of the bacteria identified in the gut microbiome belong to the Bacteroidetes and Firmicutes phyla, which include the bacteria genera of Bactereoides, Alistipes, Prevotella, Porphyromonas, Clostridium, Dorea, Faecalibacterium, Eubacterium, Ruminococcus and Lactobacillus [21]. Humans can be divided into three different enterotypes. These enterotypes are defined by the abundance of bacteria from the different genus, i.e. Bacteroides, Prevotella or Ruminococcus, and each enterotype possess a different biosynthetic pattern [22].

The indigestible components of the diet, including plant-based polysaccharides, resistant starches and mucin, which reach the colon undergo sequential degradation by different classes of the resident bacteria. The first step in this process is conversion of the complex carbohydrates to monosaccharides and oligosaccharides by Bacteroides which secrete glycan degrading enzymes. The monosaccharides and oligosaccharides subsequently undergo primary fermentation by other bacteria, generating hydrogen, carbon dioxide, ethanol and short-chain fatty acids including acetate, butyrate, propionate and lactate. Short-chain fatty acids generated by the gut microbiome serve as a major source of energy for colonocytes. In addition, other bacteria use short-chain fatty acids as a substrate for secondary fermentation reactions. The primary actions of microbial flora on amino acids include deamination, decarboxylation and release of hydrogen sulfide from sulfur-containing amino acids (methionine and cysteine), deamination of amino acids leads to formation of ammonia and short-chain fatty acids or phenolic compounds (indol and *p*-cresol) in the case of aromatic amino acids (tyrosine, phenylalanine and tryptophan). Decarboxylation of amino acids results in formation of different amines which serve as precursors for nitrosamines.

The normal microbial flora protects the host against pathogenic microorganisms [23], confers trophic functions [24] and contributes to the energy metabolism (by facilitating absorption of indigestible complex carbohydrates) [25], the micronutrient homeostasis (production of short-chain fatty acids, various vitamins such as group B vitamins and vitamin K) and nitrogen balance (synthesis of amino acids, e.g. lysine and threonine) [26–28]. In addition, the intestinal microbiome plays a major part in shaping the immune system and in the maintenance of the immune homeostasis. In fact, germfree mice display severe immune disorders that stem from the lack of the normal post-partum programing of the immune system by the microbial flora [29, 30]. The microbiota that colonize the intestinal tract after birth play a central part in priming the physiological structure and function of lymphoid tissues by shaping the functional interactions of different components of the adaptive immune system within and beyond the lymphoid tissue in the Peyer's patches [29].

For reasons outlined above, the normal intestinal microbiome is essential for health, and changes in the composition and function of the microbiome contribute to the development and progression of numerous illnesses such as inflammatory bowel diseases, diabetes, dyslipidemia, obesity, cardiovascular diseases, cancer, allergic disorders, IgA nephropathy and chronic inflammation among others [31–36].

FACTORS THAT CAN INFLUENCE INTESTINAL MICROBIOME IN CKD

The composition and function of the microbial flora is heavily influenced by the biochemical and biophysical environment. Via several mechanisms, renal failure results in profound changes in the biochemical milieu of the alimentary tract. First, the rise in urea concentration in the body fluids leads to its massive influx into the gastrointestinal tract. Within the intestinal tract, urea is hydrolyzed by microbial urease leading to formation of ammonia [$CO(NH_2)2 + H_2O \rightarrow CO_2 + 2NH_3$]. Ammonia is, in turn, converted to ammonium hydroxide [$NH_3 + H_2O \rightarrow NH_4OH$] which raises the luminal fluid's pH and causes enterocolitis [37, 38]. Second, significant amounts of uric acid and oxalate are actively secreted into the gut's lumen by colonic epithelial cells in an adaptive response to the decline in their renal excretion [39–41]. This accounts for the relatively minor increase in plasma uric acid despite the loss of its renal excretion in advanced CKD. In addition to

changing the biochemical milieu of the gastrointestinal tract, large amounts of urea and uric acid released in the intestine serve as alternative substrates for the microbial flora which normally utilize indigestible complex carbohydrates. Third, dietary restrictions which are invariably prescribed to prevent hyperkalemia and fluid overload significantly alter the biochemical milieu of the gastrointestinal tract in the CKD population. Chief among them is restricted consumption of fruits and vegetables which are a rich source of potassium. Since fruits and vegetables are the main source of dietary fiber, their limited consumption has a deep impact on the composition, function and metabolism of the gut microbiome. Fourth, use of various phosphate-binding products, i.e. anion-exchange resins, iron-based products, calcium acetate, calcium carbonate and aluminum hydroxide which are commonly prescribed for patients with advanced CKD, must have as yet unrecognized effects on the gut microbiome. Finally, frequent use of antibiotics to treat vascular access and other infections can significantly affect microbiomes in patients with advanced CKD.

UREMIA-INDUCED CHANGES IN THE COMPOSITION OF THE INTESTINAL MICROBIOME

In an earlier study, we tested the hypothesis that by modifying the biochemical environment of the gut, uremia may change the composition of the intestinal microbial flora [42]. In this study, microbial DNA was isolated from the stool samples of a group of patients with ESRD maintained on hemodialysis and a group of age-, sex- and ethnicity-matched healthy individuals. The microbial species in the test samples were determined by a novel phylogenic microarray technique developed by our collaborators. The study showed highly significant differences in the abundance of over 200 bacterial operational taxonomic units (OTUs) belonging to 23 bacterial families between the ESRD and the healthy control groups. The OTUs from the Beutenbergiaceae, Cellulomonadaceae, Dermabacteraceae, Micrococcaceae, Catabacteriaceae, Clostridiaceae, Coprobacillaceae, Polyangiaceae, Alteromonadaceae, OM60, Enterobacteriaceae, Methylococcaceae, Halomonadaceae, SUP05, Moraxellaceae, Pseudomonadaceae, Thiotrichaceae, Xanthomonadaceae and Verrucomicrobiaceae families were markedly increased whereas those belonging to the Prevotellaceae, Lactobacillaceae and Alcaligenaceae families were markedly reduced in ESRD patients. In order to isolate the effects of uremia from those of comorbid conditions, and dietary restrictions, medications and inter-individual variations, the gut microbiome was studied in rats 8 weeks after 5/6 nephrectomy or sham operation. The CKD and control rats were fed similar diets and kept under identical conditions. Compared with the control rats the CKD rats showed significant differences in the abundance of 175 bacterial OTUs, thus confirming the effect of uremia per se on the composition of the gut microbiome. For reasons outlined above, in a subsequent study, we conducted a series of microbial genomic analyses to test the hypothesis that ESRD results in expansion of bacterial families possessing urease, uricase and *p*-cresol- and indole-forming enzymes and depletion of the bacteria possessing short-chain fatty acid forming enzymes [43]. The study revealed that among the 19 microbial families that were dominant in ESRD patients, 12 possessed urease (Alteromonadaceae, Cellulomonadaceae, Clostridiaceae, Dermabacteraceae, Enterobacteriaceae, Halomonadaceae, Methylococcaceae, Micrococcaceae, Moraxellaceae, Polyangiaceae, Pseudomonadaceae and Xanthomonadaceae), 5 possessed uricase (Cellulomonadaceae, Dermabacteraceaea, Micrococcaceae, Polyangiaceae and Xanthomonadaceae families), 3 possessed indole and *p*-cresol-forming enzymes (i.e. tryptophanase possessing families: Clostridiaceae, Enterobacteriaceae and Verrucomicrobiaceae) and 2 possessed tyrosine deaminase (Clostriadiaceae and Enterobacteriaceae). Among the four microbial families that were depleted in ESRD patients, two possessed short-chain fatty acid (butyrate) forming enzymes (Lactobacillaceae and Prevotellaceae).

The observed CKD-induced changes in the composition and function of the gut microbial flora in CKD represents a dysbiotic state which has adverse consequences. For example, disruption of the normal symbiotic relationship can lead to formation and absorption of pro-oxidant, pro-inflammatory and otherwise harmful byproducts that contribute to uremic toxicity, inflammation and cardiovascular, nutritional and other complications. Likewise, the shift in the microbiome may lead to as-yet unrecognized consequences by limiting availability of many beneficial compounds that are produced by the normal flora. The observed changes in the composition and function of the gut microbiome is further complicated by the fact that duodenum and jejunum which are only lightly colonized in the normal population become intensely colonized by aerobic and anaerobic bacteria in uremic patients [44, 45].

CONTRIBUTION OF THE GUT MICROBIOME TO THE UREMIC MILIEU

The changes in the biochemical environment of the gut and the composition and function of the intestinal microbiome can lead to generation and translocation of products which can potentially contribute to local and systemic inflammation, malnutrition, uremic toxicity and other complications in patients with advanced CKD. This assumption is confirmed by the results of a study by Aronov *et al.* [46] who compared plasma solutes in healthy individuals with those found in hemodialysis patients with intact colons and hemodialysis patients who had undergone total colectomy. Employing high-resolution mass spectrometry, these investigators verified the colonic origin of a number of well-known uremic toxins including indoxyl sulfate and *p*-cresol sulfate as well as numerous as-yet unidentified compounds in the plasma of patients with ESRD by comparing data from ESRD patients who had undergone colectomy with those found in patients with intact colon and healthy persons. The study identified over 30 mass spectroscopic features in the plasma from ESRD patients with intact colons which were either absent or were present in much lower levels in patients whose colons had been removed. Almost all of these compounds were significantly more abundant in plasma from the ESRD patients than in their healthy control counterparts,

and as such represented uremic solutes. The study further verified the colonic origin of the well-known uremic toxins, *p*-cresol sulfate and indoxyl sulfate. These findings provided irrefutable evidence for the colonic microbes as the main source of numerous uremic solutes many of which have not yet been identified. Trimethylamine-*N*-oxide (TMAO) is a gut-derived toxic metabolite which is generated by release of cholin from phosphatidylcholine and its conversion to trimethylamine by the gut microbial flora. Trimethylamine is subsequently absorbed and converted to TMAO by monooxygenases in the liver. Elevated levels of TMAO have been linked to cardiovascular disease [47–49] in humans and foam cell formation and atherosclerosis in experimental animals [50]. Plasma TMAO level is elevated in CKD and ESRD patients [51, 52]. However, its association with morbidity and mortality has not been evaluated in this population and awaits investigation. It should be noted that unlike the protein-bound toxic metabolites such as indoxyl sulfate and *p*-cresol sulfate, TMAO is efficiently removed by dialysis.

In addition to production of numerous solutes, fermentation of nutrients by the gut microbial flora generate a variety of organic and inorganic gases the nature and the abundance of which is determined by the available substrates and the properties of the microbial flora and can be detected in the expired breath, blood and feces. The uremia-induced changes in the composition and function of the gut microbiome and its biochemical milieu as well as dietary and medicinal interventions can significantly alter the nature and abundance of these gaseous metabolites. Marked elevation of breath ammonia content has long been recognized as a marker of renal failure. In addition, recent studies have revealed significant changes in exhaled breath organic gases in CKD. In an earlier study, we found over 50 organic gases in the exhaled breath and 36 gases in cultured feces of rats. Many of the organic gases found in the expired breath were also found in the gases emitted from the cultured feces pointing to the gut microbial flora as their likely origin. Comparison of the data between CKD and healthy control rats revealed significant increase in isoprene and a significant reduction in pentanal, hexanal and heptanalin in exhaled breath and a marked increase in dimethyldisulfide, dimethyltrisulfide and two thioesters emitted from cultured feces in CKD rats [53].

One of the most abundant uremic gaseous metabolites is ammonia which is the byproduct of the hydrolysis of urea by urease possessing intestinal bacteria. In fact, the smell of ammonia in the breath of patients was an ancient diagnostic biomarker of uremia. Recent studies conducted in our laboratories have revealed the central role of urea-derived ammonia and ammonium hydroxide in the pathogenesis of the CKD-induced disruption of the intestinal epithelial barrier structure and function [54]. The disruption of epithelial barrier leads to local inflammation [37, 38], and inflammation leads to further disruption of the epithelial barrier, forming a vicious cycle [55].

In addition to their toxic metabolites, structural components of the gut microbial flora have been found in the blood of the uremic humans and animals. Lipopolysaccharide (LPS), otherwise known as endotoxin, is a component of the cell wall of the Gram-negative bacteria. Via binding to the Toll-like receptor-4 (TLR4) on endothelial cells, monocyte and macrophage LPS results in oxidative stress and inflammation by activating NF-κB and AP-1 and generation of inflammatory cytokines, chemokines, adhesion molecules and production of reactive oxygen, halogen and nitrogen species. CKD is commonly associated with endotoxemia, the severity of which correlates with the intensity of the prevailing systemic inflammation [56–59]. In addition to endotoxin, gut-derived microbial DNA is frequently found in the plasma from patients and animals with CKD indicating their translocation from the gut lumen in the systemic circulation [60–65]. As described later in this review, this is caused by the uremia-induced disruption of the gut epithelial barrier which enables entry of endotoxin and other microbial components into the systemic circulation.

The adverse effects of the harmful products of the dysbiotic microbial flora are compounded by the reduction of numerous useful byproducts of the normal microbiota. Chief among such products are short-chain fatty acids which are essential for the integrity of colonocytes and the growth of anti-inflammatory regulatory T lymphocytes. In fact, short-chain fatty acids produced by the gut bacteria have been recently shown to protect the kidney against ischemia reperfusion injury and mitigate the associated oxidative stress and inflammation in experimental animals [66]. In summary, the combination of uremic milieu and dietary restrictions work in concert to transform the gut microbiome from the normal symbiotic to a dysbiotic state. This leads to increased production of microbial toxic products, reduced production of essential micronutrients and local and systemic inflammation. Therefore strategies aimed at improving the biochemical milieu of the gut and raising the supply of the substrates that support the growth of the symbiotic organisms may prove effective in enhancing the well-being of the CKD populations.

THE STRUCTURE AND FUNCTION OF THE NORMAL INTESTINAL EPITHELIAL BARRIER

Although the gastrointestinal tract is anatomically located in the center of the body it is, in fact, an extension of the external environment. For this reason the intestinal epithelium serves as a barrier against entry of microorganisms, harmful microbial products and other noxious compounds into the internal milieu. The structure of the gastrointestinal barrier consists of the apical membrane of the intestinal epithelial cells and the apical junctional complex which seals the gap between the adjacent epithelial cells. While the apical membrane of the epithelial cells regulates passive and active transcellular transport of solutes, the apical junctional complex regulates paracellular transport of solutes, and water and serves as the barrier against paracellular permeation of the luminal contents of the intestinal tract [67]. The apical junctional complex consists of the tight junction which is its most luminal component and the subjacent adherens components. The tight junction consists of the adhesive transcellular proteins (occludin and claudin families); the actin-binding cytosolic proteins (the zonula occludens [ZO] protein family) and the perijunctional ring of actin and myosin. The transmembrane proteins link the plasma

membranes of the adjacent cells to form the barrier against diffusion of fluids and solutes; the ZO proteins serve as the anchor and regulate the organization of the apical junction complex; and the perijunctional ring of actin and myosin modulate the structure and function of the tight junction and regulate paracellular permeability [68]. The gut epithelial tight junction prevents the influx of microorganisms and noxious substances such as microbial toxins, antigens, digestive enzymes and degraded food products to the sub-mucosal tissue and the internal milieu.

IMPAIRMENT OF INTESTINAL BARRIER FUNCTION IN UREMIA

Several observations have provided indirect evidence of intestinal barrier dysfunction in patients and animals with advanced CKD. These include (i) presence of endotoxemia in the absence of clinical infection in the uremic patients [56–59] which is most likely derived from the gastrointestinal tract; (ii) increased intestinal permeability to high-molecular weight polyethylene glycols in the CKD patients and animals [69, 70]; (iii) demonstration of the gut bacteria and their DNA fragments in the intestinal wall, and mesenteric lymph nodes of the uremic but not normal rats [63] and detection of the gut-derived microbial DNA in the blood of hemodialysis-treated and -untreated ESRD patients [60–65] pointing to translocation of the gut microbial products to the systemic circulation in uremia, and (iv) histological evidence of chronic inflammation throughout the gastrointestinal tract, i.e. esophagitis, gastritis, duodenitis, enteritis and colitis in ESRD patients maintained on hemodialysis [38]. Together these observations in humans and animals provide compelling evidence supporting the impairment of the intestinal barrier function in advanced CKD, a phenomenon which can contribute to the pathogenesis of systemic inflammation, in this population [71].

EFFECT OF UREMIA ON INTESTINAL EPITHELIAL TIGHT JUNCTION

In view of the role of the tight junction as the critical component of the epithelial barrier structure and function, the observed increase in intestinal permeability in uremia could be due to impairment of the tight junction complex. To explore this possibility in a series of studies, we determined the abundance and localization of protein constituents of the tight junction complex in the colonic tissue of rats rendered uremic by subtotal nephrectomy or adenine-induced chronic tubulointerstitial nephritis [72]. Using immunohistology and western blot analyses we found marked depletion of claudin-1, occludin and ZO1 proteins in the colonic mucosa of the CKD groups compared with the corresponding healthy control animals. The reduction in the abundance of the measured proteins was accompanied by their normal or elevated mRNA expression pointing to a post-transcriptional or post-translational mechanism. Disruption of the colonic epithelial tight junction apparatus in the CKD animals was associated with heavy infiltration of mononuclear leukocytes in the lamina propria and marked thickening of the colonic wall. In a follow up study, we found depletion of the tight junction proteins throughout the gastrointestinal tract including stomach, jejunum and ileum [73]. These finding unraveled the underlying mechanism responsible for the previously demonstrated impairment of the intestinal barrier function and endotoxemia in humans and animals with advanced CKD.

MECHANISMS OF UREMIA-INDUCED DISRUPTION OF THE GASTROINTESTINAL EPITHELIAL TIGHT JUNCTION

In an attempt to discern the mechanism(s) of uremia-induced disruption of the intestinal epithelial tight junction, we conducted a series of *in vitro* experiments using human colonic epithelial cells seeded on Transwell plates to form an impermeable polarized monolayer that simulates *in vivo* condition. The first of these *in vitro* studies [74] tested the hypothesis that CKD-induced disruption of the intestinal epithelial tight junction is caused by the retained uremic toxins or metabolites. The study further aimed to determine whether the inciting metabolite(s) are removable by dialysis. To this end, we compared the effects of the incubation of the fully polarized cultured human colonocytes in media containing pre-dialysis and post-dialysis plasma from ESRD patients as well as plasma from healthy control individuals. Compared with the control plasma, incubation in media containing ESRD patients' pre-dialysis plasma resulted in a marked drop in transepithelial electrical resistance indicating increased permeability of the epithelial monolayer. The rise in the epithelial monolayer permeability in response to the exposure to patients' pre-dialysis plasma was accompanied by the heavy losses of the transcellular and intracellular protein constituents of the tight junction. The magnitude of the epithelial barrier damage and dysfunction was significantly less pronounced in cells exposed to the post-dialysis than pre-dialysis plasma. Taken together, these observations revealed the role of some dialyzable uremic-retained product(s) as the cause of the disruption of intestinal epithelial barrier in CKD [75].

In a follow up study, we explored the possibility that the depletion of the intestinal epithelial tight junction proteins and the resulting barrier dysfunction in CKD may be in part mediated by the heavy influx of urea into the gastrointestinal tract, its conversion by microbial urease to ammonia [$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$] and formation of ammonium hydroxide [$NH_3 + H_2O \rightarrow NH_4OH$], which is a caustic compound capable of dissolving proteins. To test this hypothesis, we incubated the fully polarized cultured human colonocytes in the culture media containing urea at clinically relevant concentrations. In order to simulate the presence of microbial flora in a separate experiment, cells were treated with urea plus urease in the culture media. The study showed a modest urea concentration-dependent fall in transepithelial electrical resistance which was coupled with a significant reduction of the tight junction proteins. The presence of urease dramatically amplified the effects of urea leading to dissipation of transepithelial electrical resistance, near total loss of the tight junction proteins and detachment of the monolayer [54]. These observations documented, for the first time, that impairment of the

intestinal barrier function is, at least in part, caused by urea which has been generally considered to be a relatively harmless uremic retained metabolite. The breakdown of the gut epithelial tight junction and resultant translocation of endotoxin and microbial fragments and other luminal noxious products in the sub-epithelial tissue promotes local inflammation as shown in the uremic humans and experimental animals (Figure 1). Inflammation, in turn, amplifies epithelial barrier disruption by triggering activation (phosphorylation) of myosin light chain kinase leading to intracellular retraction and proteasomal degradation of claudins and occludin and hence further disruption of the barrier structure. The contribution of local and systemic inflammation to the pathogenesis of CKD-induced disruption of the intestinal epithelial tight junction was recently demonstrated by the favorable response to attenuation of inflammation with an Nrf2 inducer in CKD rats [55].

OTHER FACTORS CONTRIBUTING TO INTESTINAL BARRIER DYSFUNCTION IN CKD

Edema of the intestinal wall and bowel ischemia as seen in patients with uncompensated congestive heart failure, and those with hepatic cirrhosis and portal hypertension can impair gut epithelial barrier and cause endotoxemia and systemic inflammation [75–79]. Congestive heart failure, fluid overload and generalized edema are common complications of CKD and can, therefore, contribute to the impairment of the gastrointestinal epithelial barrier function. In addition, excessive use of diuretics in CKD patients and aggressive ultrafiltration by hemodialysis in ESRD patients frequently result in episodes of hypotension which can lead to bowel ischemia in this population [80]. Thus the uremia-induced intestinal barrier dysfunction and the resulting systemic inflammation are intensified in the presence of bowel edema or ischemia. In addition, the gastrointestinal micro-bleed caused by systemic anticoagulation used with each hemodialysis treatment combined with uremic platelet dysfunction and high incidence of angiodysplasia in this population [81], can affect the integrity of the gut epithelial barrier structure and alter the microbial flora which is highly sensitive to the changes in the available iron pool. In confirmation of previous studies, ESRD groups studied by Shi *et al.* [64] had plasma endotoxin concentrations far greater than those in the dialysis solution. This observation refuted the previously held notion that the dialysis solution is the source of the post-dialysis rise in plasma endotoxin, a phenomenon most likely due to the aforementioned transient bowel ischemia.

STRATEGIES THAT MAY ATTENUATE UREMIA-INDUCED INTESTINAL BARRIER DISRUPTION AND MICROBIAL DYSBIOSIS

Based on the observations cited above, some of the following approaches may prove effective in attenuating the alteration of microbiome and intestinal epithelial barrier disruption and hence systemic inflammation and uremic toxicity in the CKD population:

Urea and other uremic toxin reducing strategies

The studies cited above have identified urea as a major mediator of the uremia-induced intestinal barrier disruption [54]

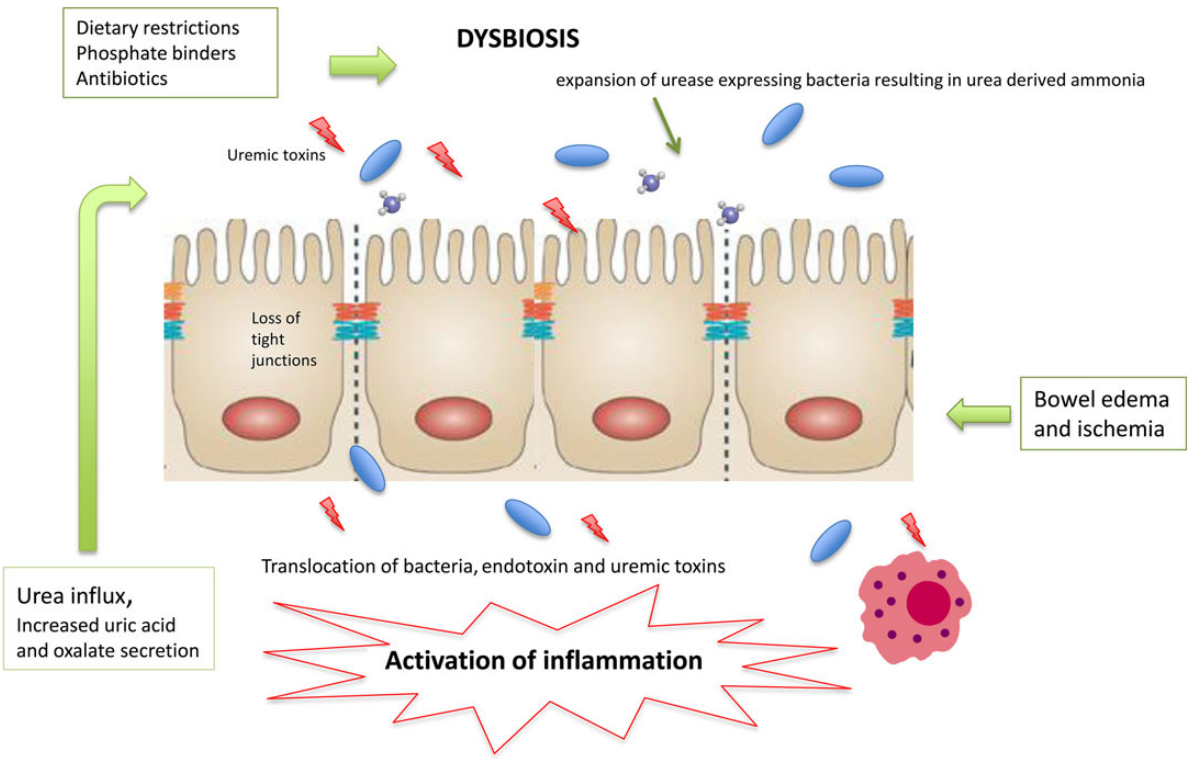

FIGURE 1: Effect of CKD on the intestinal microbiome and epithelial barrier and resulting inflammation.

and a likely mediator of altered microbiome [43, 82]. Therefore, strategies aimed at lowering urea levels may prove useful in improving systemic inflammation in patients with advanced CKD. This supposition is supported by the previously documented salutary effect of urea-lowering strategies such as the use of keto analogs of amino acids [83], low protein diet and possibly longer, more frequent and less aggressive dialysis regimens in CKD patients. In addition, the reduction of urea burden may have a potentially favorable impact on the structure and function of the gut microbiome which is profoundly affected by heavy influx of urea.

High-fiber diet

As noted above, one of the major causes of the altered composition and function of the intestinal microbiome in patients with advanced CKD is dietary restriction of potassium-rich fruits and vegetables to prevent hyperkalemia. Fruits and vegetables are the main sources of the indigestible complex carbohydrates which are the principal substrates for the gut's symbiotic microbiota. These bacteria convert the dietary fiber to short-chain fatty acids which, as noted above, are essential nutrients for the colonic epithelial cells and the regulatory T lymphocytes. The regulatory T lymphocytes are essential for the maintenance of the immunological self-tolerance and limitation of the inflammatory response [84] and their population is markedly reduced in the ESRD population [85, 86]. As mentioned above, the population of bacteria capable of forming short-chain fatty acids is markedly reduced in ESRD patients [43]. Reduced dietary intake of fermentable fiber may play a major part in depletion of the short-chain fatty acid-making bacteria, a phenomenon which can, in part, account for the reduction of the regulatory T lymphocytes and CKD-associated systemic inflammation. To test this hypothesis, in a recent study, Sprague Dawley rats with adenine-induced CKD were fed diets supplemented with amylopectin (low-fiber control) or high fermentable fiber (amylose maize-resistant starch, HAM-RS2) for 3 weeks. The CKD rats fed the low-fiber diet exhibited reduced creatinine clearance, interstitial fibrosis, inflammation, tubular damage, disruption of colonic epithelial tight junction, upregulation of inflammatory, oxidative and fibrotic pathways and impairment of the Nrf2 pathway. Consumption of the high-fiber diet significantly attenuated disruption of the colonic epithelial tight junction, oxidative stress, inflammation and severity of renal dysfunction and pathological lesions [87]. In addition, dietary fiber (resistant starch) supplementation has been shown to significantly reduce serum concentration of uremic toxins, indoxyl sulfate and *p*-cresol sulfate, in ESRD patients maintained chronic hemodialysis [88]. Taken together these observations demonstrated the potential deleterious effects of low-fiber diet and salutary actions of fiber-rich diet on CKD progression.

Intestinal alpha-glycosidase inhibition

Another strategy that can be used to increase delivery of carbohydrates to colon is administration of an inhibitor of small intestinal alpha-glycosidase such as acarbose. By limiting the breakdown of digestible carbohydrates to absorbable monosaccharides, use of this agent enables delivery of undigested carbohydrates to the colon for fermentation by the resident bacteria. In an earlier study Evenepoel *et al.* [89] it was shown that administration of acarbose significantly reduced *p*-cresol levels in the urine, plasma and feces of a group of individuals with normal renal function. Based on these observations they suggested that administration of acarbose may lower the burden of the gut microbial-derived protein-bound uremic toxins in patients with CKD.

Oral adsorbents

Activated charcoal has been widely used as a decontaminant in cases of acute poisoning and as an effective degassing agent to reduce abdominal bloating and flatulence. In addition, AST-120, a highly potent activated charcoal preparation has been shown to markedly reduce plasma concentration of indoxyl sulfate and *p*-cresol sulfate [90, 91] which are well-known uremic toxins produced by the gut microbial flora [46, 92, 93]. Moreover, administration of AST-120 in animal models of chronic renal disease has been shown to reduce oxidative stress and inflammation and retard progression of renal disease [94–97]. The salutary effects of AST-120 have been primarily attributed to its ability to limit formation and absorption of indoxyl sulfate [97, 98]. As noted above, our recent study demonstrated the role of urea-derived ammonia and ammonium hydroxide as a major cause of the uremia-induced intestinal epithelial barrier destruction/dysfunction [54]. Given the ability of activated charcoal to avidly adsorb ammonia, AST-120 can potentially attenuate the severity of uremia-induced intestinal barrier disruption. If true, the widely reported salutary effects of AST-120 in reducing inflammation and oxidative stress must be, in part, due the preservation of the intestinal epithelial barrier function and structure. This supposition was confirmed by our recent study [99] which tested the hypothesis that administration of AST-120 may attenuate uremia-induced disruption of the intestinal epithelial tight junction apparatus in animals with chronic renal failure. The study revealed marked elevations of plasma endotoxin, IL-6, TNFα, MCP-1, CINC-3, L-selectin, ICAM-1 and malondialdehyde levels, and heavy depletions of colonic epithelial TJ proteins in the untreated CKD rats. Administration of AST-120 resulted in partial restoration of the epithelial TJ proteins and reduction in plasma endotoxin and markers of oxidative stress and inflammation [99].

Strategies aimed at minimizing bowel edema and preventing bowel ischemia

As noted above, bowel edema and ischemia result in epithelial barrier impairment which can lead to endotoxemia and systemic inflammation. Fluid overload and congestive heart failure which are common complications of advanced CKD can lead to generalized edema including edema of the visceral tissues. Conversely excessive use of diuretics in CKD patients and aggressive ultrafiltration by hemodialysis in ESRD patients frequently result in episodes of hypotension which can lead to bowel ischemia in this population. Thus the uremia-induced intestinal barrier dysfunction and the resulting systemic inflammation are intensified in presence of both fluid overload and volume depletion. Therefore, it is essential to prevent or minimize these events by limiting dietary fluid and sodium intake, and

by longer, more frequent and less intense dialysis to minimize fluid retention and the need for aggressive ultrafiltration.

Probiotics

Given the role of the altered composition of the gut microbial flora in the pathogenesis of uremic toxicity and systemic inflammation, several studies have explored the effect of oral administration of certain bacterial species on the burden of targeted uremic toxins and metabolites [100–102]. Most of these preparations included urease-possessing bacteria intended to lower urea levels. Except for marginal reduction in urea level, no significant reduction in other uremic toxins or metabolites was observed. Moreover a randomized clinical trial of Renadyl® failed to reduce plasma concentration of protein-bound uremic toxin or markers of systemic inflammation and did not improve quality of life parameters [102]. This is not surprising since the alteration of the structure and function of the gut microbial community is caused by a combination of the uremia-induced changes in the biochemical/biophysical environment of the intestine and dietary and medicinal regimens which create an unfavorable milieu for the symbiotic microbiota. Consequently attempts to restore the desired microbiome by introducing favorable microorganism without simultaneously improving the gut's biochemical milieu will be futile. The reason that administration of urease-possessing bacteria has shown marginal reduction in urea level is that heavy influx of urea creates a hospitable milieu for expansion of these bacteria. In fact, as described above, the population of urease-possessing bacteria is greatly expanded in CKD patients [53]. Moreover, conversion of urea by these organisms to ammonia and ammonium hydroxide, which are responsible for destruction of intestinal epithelial barrier, heightens inflammation and as such is detrimental.

FULL REVIEW

CONCLUSIONS

CKD results in profound changes in the composition of the gut microbiome and disruption of the intestinal epithelial barrier structure and function. These abnormalities lead to generation and absorption of noxious and toxic byproducts which contribute to the systemic inflammation, uremic toxicity, malnutrition and various other morbidities in this population. Strategies aimed at lowering the urea level, minimizing fluid overload and avoiding volume depletion, as well as use of oral adsorbents and high-fiber diet may enhance the well-being of this vulnerable population by attenuating uremia-induced alteration of the gut microbiome and epithelial barrier disruption.

CONFLICTS OF INTEREST STATEMENT

None declared.

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