

The gut–kidney–heart axis in chronic kidney disease

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Received: August 10, 2019

Accepted: August 21, 2019

The recent explosion of scientific interest in the gut microbiota has dramatically advanced our understanding of the complex pathophysiological interactions between the gut and multiple organs in health and disease. Emerging evidence has revealed that the gut microbiota is significantly altered in patients with chronic kidney disease (CKD), along with impaired intestinal barrier function. These alterations allow translocation of various gut-derived products into the systemic circulation, contributing to the development and progression of CKD and cardiovascular disease (CVD), partly mediated by chronic inflammation. Among potentially toxic gut-derived products identifiable in the systemic circulation, bacterial endotoxin and gut metabolites (e.g., p-cresyl sulfate and trimethylamine-N-oxide) have been extensively studied for their immunostimulatory and atherogenic properties. Recent studies have also suggested similar biological properties of bacterial DNA fragments circulating in the blood of patients with CKD, even in the absence of overt infections. Despite the accumulating evidence of the gut microbiota in CKD and its therapeutic potential for CVD, the precise mechanisms for multidirectional interactions between the gut, kidney, and heart remain poorly understood. This review aims to provide recent evidence on the associations between the gut microbiota, CKD, and CVD, and summarize current understanding of the potential pathophysiological mechanisms underlying the “gut–kidney–heart” axis in CKD.

Keywords: bacterial DNA, cardiovascular disease, chronic kidney disease, constipation, end-stage renal disease, microbiome, permeability, uremic toxins

Introduction

Chronic kidney disease (CKD) is a significant public health problem worldwide with increasing prevalence and poor outcomes such as cardiovascular disease (CVD), end-stage renal disease (ESRD), and mortality, consuming a disproportionate amount of financial resources (16, 49). The increased risk of CVD in patients with CKD is not fully accounted for and supported by traditional cardiovascular (CV) risk factors, such as dyslipidemia, hypertension, and diabetes mellitus (111), and low-grade chronic inflammation, one of the so-called non-traditional CV risk factors, has been implicated as a primary shared risk factor of the excess risk of CVD in this unique population (2, 9, 48). Considerable efforts have been

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established to reduce the inflammatory load in patients with CKD primarily by targeting its known causative factors; however, they have been proven largely unsuccessful (39). Hence, the substantial disease burden attributable to inflammation remains unresolved.

Over the past few decades, the characteristics and functions of gut microbiota have been extensively studied, and a number of studies have revealed that the alteration of gut microbiota (i.e., gut dysbiosis) plays a central role in the pathogenesis of many diseases including CKD and CVD, often referred to as the “gut–kidney” and “gut–heart” axes, respectively (12, 74). Several factors such as impaired intestinal barrier and gut-derived toxic products have been suggested to contribute to these relationships partly mediated by systemic inflammation (12), suggesting that the gut is a potential cause of CKD-related complications and more importantly could serve as a novel therapeutic target for CVD in CKD (73). However, the precise mechanisms for the possible multidirectional interactions between the gut, kidney, and heart remain poorly understood.

In this review, we describe recent evidence on the association between the gut microbiota, CKD, and CVD and summarize current understanding of the potential pathophysiological mechanisms underlying the “gut–kidney–heart” axis in CKD (Fig. 1).

Composition of the Gut Microbiota

The human gastrointestinal tract harbors a complex community of more than 100 trillion individual organisms (106). The gut microbiota encodes at least 150-fold more genes than the human genome, which is also known as the gut microbiome (94). The introduction of high-throughput microbial DNA sequencing technologies has enabled profiling of diverse microbial

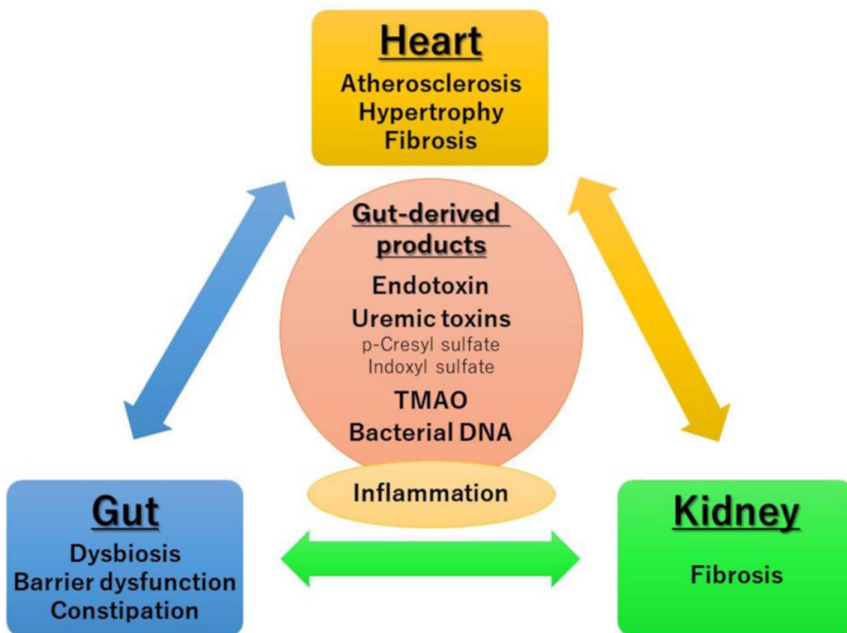


Fig. 1. Schematic representation of the gut–kidney–heart axis. TMAO: trimethylamine-N-oxide

communities in the gut, providing evidence that the overall composition and abundance of the gut microbiota vary significantly among geographically separated human populations, while dominant members of the gut microbiota are similar across the globe and are independent of race and gender (53). In general, the gut microbiota in healthy adults is dominated by two bacterial phyla, Firmicutes and Bacteroidetes, out of over 50 bacterial phyla; and other bacterial phyla include Actinobacteria, Proteobacteria, Cyanobacteria, and Fusobacteria (17, 93). Given a constant diet, the composition of the gut microbiota remains stable over time within an individual (20), which however can be easily altered by changes in diet (14).

Host–Microbiota Interaction in Health and Disease

For millions of years, the gut microbiota has coevolved with the human host and their proper interactions provide the host with various symbiotic benefits. Under physiological conditions, the gut microbiota participates in a variety of metabolic activities and has thus been recognized as a metabolically active endogenous organ in itself (74). These complementary metabolic activities include synthesis of certain vitamins (30), degradation of undigestible plant polysaccharides and oxalates (32), and biotransformation of conjugated bile acids (34). Therefore, changes in the composition and function of the gut microbiota, such as an increase in Firmicutes to Bacteroidetes ratio, have indeed been associated with numerous disease states including hypertension (1), heart failure (46), CVD (8), and CKD (65).

Short-chain fatty acids (SCFAs) and host physiology

One of the mechanisms by which the alteration of gut microbiota is linked to these conditions is through reduced production of SCFAs, such as acetate, propionate, and butyrate. SCFAs are the products of bacterial fermentation of plant-derived carbohydrates mainly by Bacteroidetes and Firmicutes (50), and play a vital role in regulating the homeostasis of human body and progression of diseases (64). Specifically, SCFAs serve as important energy resources for colonic epithelial cells (36) and maintain the integrity of the epithelial barrier function by regulation of tight junction proteins including occluding, claudin-1, and zonula occludens-1 (104). Furthermore, SCFAs can protect the intestinal epithelium via enhancement of the modulation of host immune responses by two major SCFA signaling mechanisms: inhibition of histone deacetylases (HDACs) and activation of a certain G protein-coupled receptors (GPRs) (33, 89, 95). The HDACs are enzymes that prevent DNA transcription and thereby regulate gene expression. Inhibition of HDACs in colonocytes and mucosal immune cells by SCFAs could therefore lead to suppression of the nuclear factor-kappa beta (NF- κ B) in several types of cells in the mucosal immune system, thereby influencing transcription of inflammatory-associated genes, including those that code for proinflammatory cytokines [e.g., interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α)] (10, 110). In fact, increased NF- κ B activation was found in colonic biopsies from patients with Crohn's disease, which was inhibited by applying butyrate to the specimen, accompanied by a reduction in the release of several proinflammatory cytokines (27, 77). Meanwhile, the GPRs, particularly GPR43, GPR41, and GPR109A, have been identified as colonic receptors for SCFAs and play a major role in the regulation of inflammatory processes partly through suppressing the expression of NF- κ B and promoting the differentiation of colonic regulatory T cells (79, 92). Of interest, recent studies have shown that SCFAs influence blood pressure regulation partly through GPR41, which is also expressed in the endothelium and is shown to mediate a hypotensive response (60, 68). This

is also supported by the finding that the GPR41 gene-deleted mice developed isolated systolic hypertension with increased vascular fibrosis, increased pulse wave velocity, and cardiac hypertrophy compared with wild-type littermates (60).

Taken together, SCFAs serve as key regulators for modulation of gut integrity, energy homeostasis, immune function, and blood pressure response. Therefore, a state of reduced abundance of SCFA-producing taxa in the gut could in turn negatively affect various disease conditions, such as inflammatory bowel disease, colorectal cancer, hypertension, and CVD (5, 21).

The Gut Microbiota in CKD

Emerging evidence has revealed that, compared with healthy individuals, patients with CKD show a significant alteration of the gut microbiota, with increased numbers of specific microbial taxa of both aerobic and anaerobic bacteria (78). In a study comparing the composition of the gut microbiota between CKD and control rats, a significant difference was found in the abundance of 175 bacterial operational taxonomic units (OTUs), which are microbial genomic sequences clustered by sequence similarity, with notable decreases in certain families of the Bacteroidetes and Firmicutes phyla, especially *Lactobacillaceae* and *Prevotellaceae* families, in the CKD rats (99). The study also revealed a significant difference in the abundance of 190 bacterial OTUs between patients with ESRD and healthy individuals (99). Other studies have shown that the number of *Enterobacteriaceae* (especially *Enterobacter*, *Klebsiella*, and *Escherichia*), *Enterococci*, and *Clostridium perfringens* was significantly higher in the stool of patients with ESRD than healthy controls (29, 103).

One of the contributing factors to the gut dysbiosis in CKD may be the uremic milieu. Bacterial urease in the gut, for example, hydrolyzes urea and produces large quantities of ammonia and ammonium hydroxide, which increase luminal pH and result in the alteration of gut microbial compositions (98, 108). Other factors potentially contributing to the altered gut microbiota in CKD include restricted intake of dietary fiber (35), medication use (e.g., antibiotics, phosphate binders, and iron) (42, 47, 96), slow intestinal transit time (109), and metabolic acidosis (24). It may be important to note that most of these factors have also been suggested as causative factors for the intestinal barrier dysfunction, which has recently been increasingly recognized in patients with CKD (3, 55, 91).

Intestinal Barrier Dysfunction in CKD

Intestinal barrier dysfunction and bacterial translocation have been well described in certain diseases, such as inflammatory bowel disease (52), portal hypertension (51), and heart failure (101). Emerging evidence has revealed that the intestinal epithelial barrier is altered both structurally and functionally in patients with CKD, resulting in increased gut permeability (55). Several factors have been suggested to account for the gut barrier dysfunction in CKD, including gut dysbiosis (41, 87), slow intestinal transit time (109), low dietary fiber intake (44), metabolic acidosis (41), gut ischemia and edema (87), iron therapy (102), and frequent use of antibiotics (15). Urease enzyme produced by certain microbiota families, such as *Enterobacteriaceae*, for example, hydrolyzes urea in the gut, leading to the formation of ammonium hydroxide. The high amount of ammonium hydroxide could then induce disruption of the intestinal epithelial tight junctions, characterized by the depletion of

occludin, claudin-1, and zona occludens proteins (99). Recent evidence has also revealed that dysfunction of tight junction proteins results in reduced expression of nuclear factor erythroid 2-related factor 2 (NRF2), a regulator of antioxidant defense genes, thus elevating levels of oxidative stress in the uremic milieu (45). The resultant increase in gut permeability, in turn, allows translocation of gut-derived toxins, bacterial fragments, and intact bacteria through the bowel wall into the systemic circulation, which has been considered as a key contributor to the activation of host inflammatory responses, potentially leading to excess morbidity and mortality in CKD (100).

Gut-Derived Products and CVD in CKD

Among possible gut-derived products identifiable in the systemic circulation, bacterial endotoxin [i.e., lipopolysaccharide (LPS)], and gut metabolites [e.g., p-cresyl sulfate and trimethylamine-N-oxide (TMAO)] have been extensively studied for their immunostimulatory and atherogenic properties (19, 90, 107). Recently, using the technique of bacterial DNA amplification, similar biological properties have also been recognized for circulating bacterial DNA fragments among patients with CKD, even in the absence of overt infections (87).

Bacterial endotoxin and CVD in CKD

Bacterial endotoxin, a LPS constituent of the external cell wall of most Gram-negative bacteria, is continuously produced in the gut and translocated into the systemic circulation across the intestinal barrier (54, 63). Patients with CKD are characteristically exposed to significant endotoxemia due to altered gut permeability and/or endotoxin contamination of dialysis water (23, 72). A previous study showed that the levels of circulating endotoxin increased with advancing CKD stages, with the highest levels observed in patients receiving dialysis (54). In addition, accumulating evidence has revealed the strong association between endotoxemia and systemic inflammation, which has been implicated in the development and progression of atherosclerosis and subsequent CVD in CKD (22). Toll-like receptor 4 (TLR4), which is expressed on various tissues including cells of the innate immune system, smooth muscle, and endothelial cells and binds to circulating endotoxin, plays a central role in the inflammatory process (63). Endotoxin-mediated signaling through TLR4 stimulates IL-1 receptor-associated kinase via mutant myeloid differentiation primary response 88 and myeloid differentiation protein 2, and the subsequent recruitment of TNF receptor-associated factor 6 leads to the activation of the NF- κ B and the release of various proinflammatory cytokines (59, 88). These inflammatory responses result in endothelial cell injury, transformation of macrophages to foam cells, and procoagulant activity, and could consequently contribute to the development and progression of atherosclerosis (11).

Microbiota metabolites and CVD in CKD

p-Cresyl sulfate and indoxyl sulfate. Protein fermentation by certain gut bacteria results in the generation of various metabolites, some of which exert toxic effects and thus are called uremic toxins (25). p-Cresyl sulfate and indoxyl sulfate are the two major gut-derived uremic toxins (31). p-Cresyl sulfate, a 188-Da protein-bound solute, originates from sulfation of the intestinally generated p-cresol, which is a colonic fermentation product of the amino acid tyrosine and phenylalanine (13). Intestinal bacteria also have tryptophanase that converts tryptophan to indole, which is subsequently absorbed and metabolized by the liver to indoxyl sulfate (13). Both p-cresyl sulfate and indoxyl sulfate are normally secreted by the proximal

tubular cells of the kidneys. However, as kidney function declines, these two uremic toxins accumulate progressively and exert deleterious effects on various tissues and organs, such as renal tubular cell damage, endothelial dysfunction, leukocyte activation, coagulation disturbances, insulin resistance, and cardiac fibrosis and hypertrophy (69, 97). p-Cresyl sulfate, for example, increases expression of DNA methyltransferases 1, 3a, and 3b isoforms that suppress Klotho gene expression in the renal tubular cells and also induces epithelial-to-mesenchymal transition-like changes through activation of the renal renin angiotensin aldosterone system/transforming growth factor-beta pathway, contributing to kidney injury and fibrosis (84, 85). In addition, it induces nicotinamide adenine dinucleotide phosphate oxidase activity and reactive oxygen species production in cardiomyocytes, enhancing cardiac apoptosis and resulting in diastolic dysfunction (28). In line with these experimental observations, several clinical studies have shown that elevated levels of p-cresyl sulfate and indoxyl sulfate are associated with higher risk of CKD progression, CVD, and mortality (4, 56).

Trimethylamine-N-oxide. TMAO is a circulating organic compound derived from the metabolism of dietary L-carnitine and choline by intestinal bacteria, which was recently found to directly promote atherosclerosis in rodents (38, 105). Although the precise mechanisms whereby TMAO induces atherosclerosis remain elusive, the prevailing hypothesis is that TMAO causes alteration of cholesterol and sterol metabolism and promotes pro-atherogenic foam cell formation in the arterial wall by increasing the expression of scavenger receptors on macrophages or by restricting reverse cholesterol transport out of these cells (38, 105). Recent clinical studies have demonstrated that elevated levels of TMAO are independently associated with future CV events in non-CKD and non-dialysis-dependent CKD populations (80). Although this association has not yet been well confirmed in the ESRD population receiving hemodialysis (81), most current evidence supports the mechanistic link between TMAO and CV outcomes.

Bacterial DNA fragments and CVD in CKD

Due to its highly conserved sequence of 16S ribosomal RNA gene in the genome, circulating bacterial DNA fragment is the most easily and consistently detectable component that could also be easily discerned from human DNA (87). In recent years, increasing attention has been paid to circulating bacterial DNA fragments for their immunostimulatory properties and novel pathogenic potential for CVD in patients with CKD, particularly among those with ESRD receiving dialysis (87). Bacterial DNA contains unmethylated cytosine-guanine dinucleotide, with two purine 5' and two pyrimidine 3' (CpG) terminations. These are structures required to interact with TLR9 (bacterial DNA receptor), which triggers a cell signaling pathway including activation of the mitogen-activated protein kinases and the NF- κ B (43). In polymorphonuclear neutrophils, bacterial DNA exerts profound effects on neutrophil functions by promoting neutrophil trafficking, inducing chemokine expression, regulating expression of adhesion molecules, enhancing phagocyte activity, and rescuing neutrophils from constitutive apoptosis (18). It also promotes the survival of mononuclear cells and induces IL-6 from those cells (62, 75). The inflammatory responses induced by bacterial DNA fragments have also been involved in endothelial injury through induction of endothelial cell apoptosis in hemodialysis patients (57). Furthermore, an experimental study has suggested a direct effect of circulating bacterial DNA fragments on the CV system via suppression of cardiac myocyte contraction (66). In fact, some observational studies have demonstrated significant associations of circulating bacterial DNA level with serum C-reactive protein and IL-6 levels and subsequent risk of CV events in patients with

ESRD (86). Of note, the association with CV events was found to be less substantial for plasma endotoxin (vs. bacterial DNA) levels, suggesting that, compared to plasma endotoxin, which is a cell wall component of only Gram-negative bacteria, circulating bDNA fragments that derive from both Gram-positive and -negative bacteria could play a more predominant role in the development of CVD (87).

Gut Microbiota-Targeted Interventions for CVD in CKD

Improved understanding of the pathological roles of gut dysbiosis has triggered interest in the development of various therapeutic interventions, which aimed at reestablishing symbiotic status of the gut microbiota or reducing absorption of gut-derived metabolites (73). These include dietary modifications (37) and the administration of probiotics (i.e., live microorganisms) (61), prebiotics (i.e., non-digestible food ingredients) (40), synbiotics (i.e., both probiotics and prebiotics) (26), oral adsorbents (e.g., AST-120) (76), and genetically engineered bacteria (71). Importantly, however, all of these previous studies have examined the therapeutic effect on surrogate measures; hence, it remains unclear whether these interventions actually reduce CV risk in patients with CKD.

As another gut microbiota targeted intervention for CVD in CKD, laxatives may be a potentially novel treatment option. In patients with CKD, the prevalence of constipation has been reported to be higher than in the general population (7). Since gastrointestinal motility and gut microbiota are closely interrelated and exert reciprocal effects on each other (70), constipation, one of the clinical forms of gut dysbiosis (67), is recently considered to be involved in the pathogenesis of atherosclerosis partly through chronic inflammation induced by bacterial endotoxins (6) and/or gut-derived metabolites (90), potentially contributing to adverse clinical outcomes (82, 83). In addition, a recent study demonstrated that lubiprostone, a chloride channel activator used for constipation treatment, ameliorated the CKD progression and the accumulation of uremic toxins by improving the altered gut microbiota in CKD mice (58). Given the high prevalence of constipation, the substantial alterations of gut microbiota in CKD and the increasing roles of the gut in the disposal of uremic toxins with declining kidney function, the administration of laxatives might be a potentially attractive gut microbiota-targeted therapeutic intervention for CVD in patients with CKD.

Conclusions

While the relationship between gut health and human disease processes has long been recognized, with the earliest evidence dating back to more than 2,000 years ago when Hippocrates famously stated that “*All disease begins in the gut,*” the recent explosion of scientific interest in the gut microbiota has dramatically advanced our understanding of the complex pathophysiological interactions between the gut and multiple organs. Emerging evidence on the association between gut microbiota, CKD, and CVD has provided novel mechanistic insight into the “gut–kidney–heart” axis, emphasizing the importance of the gut as a potential cause of CKD-related complications. Furthermore, these findings have opened new avenues for the development of personalized, gut microbiota-targeted therapeutic strategies for CVD in CKD, which if proved to be effective, will have a substantial impact on the CKD management. More in-depth basic and clinical research is needed to further

understand the pathophysiological mechanisms underlying the “gut–kidney–heart” axis to ultimately improve the outcomes of patients with CKD.

Acknowledgements

Dr. CPK is an employee of the US Department of Veterans affairs. The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as official policy or interpretation of the Department of Veterans Affairs or the US government. The results of this paper have not been published previously in whole or part.

Conflict of interest

None of the authors have relevant conflicts of interest.

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