Effect of Synbiotic Therapy on Gut–Derived Uremic Toxins and the Intestinal Microbiome in Patients with CKD

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The gastrointestinal tract houses a large community of microbes (microbiome), which plays a major role in micronutrient and immune homeostasis, energy metabolism, and host defenses against pathogens (1,2). Composition and function of the microbiome are shaped by the biochemical and biophysical conditions and the available nutrients. Renal failure and the commonly prescribed dietary and medicinal interventions profoundly alter the gastrointestinal milieu. Higher urea concentration in body fluids results in an increased influx into the gastrointestinal tract, where it is converted to ammonia ($CO[NH_2]_2 + H_2O \rightarrow CO_2 + 2NH_3$) by urease-possessing microbes. Ammonia is then converted to ammonium hydroxide ($NH_3 + H_2O \rightarrow NH_4OH$), which raises the luminal fluid pH (3). In addition, CKD causes active secretion of uric acid and oxalate into the colon (4,5). Influx of urea, uric acid, and oxalate alters the biochemical milieu of the gastrointestinal tract and facilitates domination of microbes that can use these substrates over the normal bacteria that consume indigestible carbohydrates. The effect of the uremic milieu is compounded by dietary restriction of potassium-rich products, including fruits and vegetables, to avoid hyperkalemia. Because fruits and vegetables are the major sources of indigestible complex carbohydrates (fibers) that constitute the main nutrients for the normal colonic bacteria, their limited consumption profoundly affects the gut microbiome. In addition, commonly prescribed phosphate and potassium binders can affect the microbiome. Finally, because the growth and replication of the gut microbiota are influenced by the flow rate of colonic contents, constipation, which is common in CKD, also affects colonic microbiota.

Using phylogenic microarray on the microbial DNA isolated from the stool samples of patients with ESRD and healthy controls, we found a significant difference in the abundance of close to 200 bacterial species belonging to 23 bacterial families between the two groups (6). To isolate the association with CKD from comorbid conditions, dietary restrictions, medications, and interindividual variations, we examined the gut microbiome in male rats kept under identical conditions 8 weeks after 5/6 nephrectomy or sham operation. Compared with the controls, the CKD rats showed a significant difference in the abundance of 175 bacterial species (6). These findings confirmed the importance of uremia *per se* on the composition of gut microbiome. Analysis of the microbial genomics revealed heavy expansion of bacteria possessing urease, uricase, and *p*-cresol– and indole-forming enzymes and depletion of bacteria possessing short–chain fatty acids forming enzymes (7). The CKD-induced changes in the composition and function of the gut microbiota represent a dysbiotic state that has adverse consequences. For example, increased generation of toxic solutes (*e.g.*, indoxyl sulfate [IS], *p*-cresol sulfate [PCS], and trimethylamine-*N*-oxide) and diminished production of beneficial micronutrients may contribute to systemic inflammation, CKD progression, and cardiovascular complications (8,9).

Patients with CKD and ESRD frequently exhibit endotoxemia, the magnitude of which is directly related to severity of systemic inflammation (10). The primary source of circulating endotoxin in patients with CKD and CKD animals is the gastrointestinal tract. Moreover, gut bacterial DNA fragments are found in the blood of patients with CKD not requiring dialysis and patients with ESRD maintained on dialysis and the blood, mesenteric lymph nodes, liver, and spleen of animals with reduced kidney function (11). Studies conducted in our laboratory revealed breakdown of intestinal epithelial tight junction that can accommodate the entry of endotoxin, microbial fragments, and other noxious intestinal contents to the systemic circulation (12). Subsequent studies revealed the role of ammonia and ammonium hydroxide produced *via* hydrolysis of urea by the urease-possessing bacteria in disruption of the intestinal barrier (7,13). These observations unraveled the central role of urea-derived ammonia as a major cause of systemic inflammation in CKD. In fact, treatment with oral-activated charcoal to adsorb ammonia resulted in significant reduction of endotoxemia and plasma concentration of inflammatory cytokines, chemokines, and adhesion molecules in animals with reduced kidney function (14). Disruption of the intestinal epithelial tight junction by heavy influx of urea and expansion of urease-possessing bacteria is compounded by the reduced production of short–chain fatty acids, which

compromises viability of colonic epithelial cells, because short–chain fatty acids generated from fermentation of carbohydrates by the symbiotic bacteria are the major source of nutrients for colonic epithelial cells (15). Increased formation of toxic metabolites (IS, PCS, and trimethylamine-*N*-oxide) and disruption of the intestinal epithelial barrier, changes in the gut milieu, and the resulting microbial dysbiosis play a major role in the pathogenesis of systemic inflammation, a likely important contributor to morbidity and mortality in the CKD population (16).

Recognition of the role of the altered gut microbiome in the pathogenesis of systemic inflammation in CKD has made it an attractive therapeutic target. The following approaches have been tested in humans and animals with CKD: (*1*) probiotic therapy with administration of live microbial species, (*2*) oral adsorbents to limit absorption of the microbial-derived toxins, (*3*) prebiotics to restore symbiotic and suppress dysbiotic microbes, and (*4*) a combination of prebiotics and probiotics (synbiotic).

As described in recent reviews (16,17), use of probiotics alone has proven ineffective in attenuating systemic inflammation, uremic toxicity, and adverse clinical outcomes. This is not unexpected, because changes in composition and function of the microbiota in CKD are mediated by the gut's unfavorable biochemical/biophysical milieu. Consequently, it is impossible to restore normal microbiome simply by introducing favorable microorganisms. Moreover, in many patients, urease-possessing organisms were used in a futile attempt to lower the serum urea level. This is counterproductive, because conversion of urea to ammonia by urease-possessing bacteria amplifies systemic inflammation (13). In addition, ammonia generated in the gut is transported by portal vein to the liver, where it is converted to urea, explaining the lack of significant reduction in urea level in the treated subjects.

The primary nutrients for the symbiotic colonic microorganisms are complex carbohydrates that promote their growth and generation of beneficial micronutrients for their host. In a recent randomized clinical trial, Sirich *et al.* (18) compared the effect of resistant starch (amylose) versus digestible starch (amylopectin) supplementation for 6 weeks in patients on hemodialysis. The study revealed a significant reduction in serum IS and a nonsignificant reduction of PCS in the resistant starch–treated group. Similar results have been observed with several other products in patients with CKD (17). Unfortunately, because of the short duration and possibly inadequate amounts of the prebiotics given, the effect of these treatments on clinical outcomes could not be assessed. However, a recent study showed significant attenuation of oxidative stress, inflammation, restoration of the colonic epithelial tight junction, and delayed CKD progression in rats fed a diet containing close to 50% resistant starch compared with those fed a low-fiber diet (19). Future clinical studies are needed to explore the efficacy of long-term use of high dietary fiber supplementation in the CKD population.

As described in a comprehensive review by Vanholder and Glorieux (17), a few studies have explored the effect of coadministration of pre- and probiotics on microbial–derived uremic toxins. In this issue of the *Clinical Journal of American Society of Nephrology*, Rossi *et al.* (20) report the results of a randomized, double–blind, crossover trial of coadministration of a prebiotic and a probiotic for 6 weeks separated by a 4-week washout period and changes in serum IS and PCS in patients with CKD (eGFR between 10 and 30 ml/min per 1.73 m^2). The prebiotic component consisted of a mixture of high molecular weight inulin, fructo-oligosaccharides, and galacto-oligosaccharides (7.5 g once daily during the first 3 weeks and twice daily during the final 3 weeks of the trial). The probiotic component included nine different strains of the *Lactobacillus*, *Bifidobacteria*, and *Streptococcus* genus packaged in capsules containing 45 billion CFUs in each prebiotic portion. Stool samples collected at baseline and the end of each arm of the trial were tested for the determination of microbiome profiles using 16S ribosomal RNA gene sequencing. Among 37 individuals enrolled, 31 completed the study. The synbiotic therapy significantly reduced serum PCS but not IS in all participants. However, in patients who had not received antibiotics during the trial, synbiotic therapy significantly lowered serum levels of both IS and PCS. These findings are consistent with two previous studies that showed significant reductions in IS and PCS with synbiotic therapy in patients with CKD or ESRD (21,22). Synbiotic therapy resulted in a significant increase in the relative abundance of fecal *Bifidobacterium* and a reduction of *Ruminococcaceae* bacteria. The relative change in *Bifidobacterium* abundance showed an inverse correlation with serum concentrations of free PCS and IS. Synbiotic therapy had no significant effect on eGFR, urinary Kim-1, or serum concentrations of endotoxin, biomarkers of inflammation or oxidative stress. Interestingly, the synbiotic therapy resulted in a small but significant increase in albuminuria. The underlying mechanism by which this synbiotic therapy might increase albuminuria is unknown. The serum albumin level was not provided in the paper. Rossi *et al.* (20) attributed the observed reduction of PCS level to *Bifidobacterium* spp.–driven suppression of PCS-forming bacteria. In addition, by lowering the pH of colonic contents, formation of short–chain fatty acids from fermentation of the polysaccharides (prebiotics) inhibits the enzymes involved in generation of PCS and IS precursors (19). Because of the short durations of this trial and previous trials, the effect of therapy on clinical outcomes could not be determined. In addition to the duration of therapy, the quantity of prebiotics can play a role in the clinical outcomes and the biochemical parameters (19).

Treatment with the synbiotic regimen used in the study by Rossi *et al.* (20) resulted in significant reduction of serum PCS and to a lesser extent, IS in patients with advanced CKD. Given the demonstrated association of these gut–derived uremic toxins with cardiovascular disease and CKD progression, future studies are needed to explore the effect of long-term administration of synbiotics with a higher amount of prebiotic component on clinical outcomes in CKD and ESRD populations.

Disclosures

None.

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See related article, "Synbiotics Easing Renal Failure by Improving Gut Microbiology (SYNERGY): A Randomized Trial," on pages 223–231.