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Uraemic toxicity

Editorial Comment

Gut microbiota and inflammation in chronic kidney disease patients

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Abstract

Inflammation is a multifactorial phenotype that in chronic kidney disease is associated with adverse patient outcomes. Recently, alterations in gut microbiota composition and intestinal barrier have been associated with inflammation and oxidative stress in CKD patients. Vanholder and Glorieux recently critically reviewed [*Clin Kidney J (2015) 8 (2): 168-179*] the current understanding of the role of gut microbiota in the production of uraemic toxins and the therapeutic implications. Where do we stand now? The basic mechanisms of the gut-kidney crosstalk must still be clarified. In addition, the efficacy and safety of therapeutic strategies to modulate the gut microbiota in order to decrease uraemic toxin production and inflammation in chronic kidney disease should be evaluated. Finally, an impact of such strategies on hard outcomes should be demonstrated before incorporation into routine clinical practice.

Keywords: chronic kidney disease; gut microbiota; inflammation

Inflammation is a multifactorial phenotype during chronic kidney disease (CKD). Many factors such as decreased clearance of proinflammatory cytokines, oxidative stress, metabolic acidosis, infections, dialysis access problems and obesity contribute to inflammation [1]. More recently, alterations in gut microbiota composition and intestinal barrier have been shown to be associated with inflammation and oxidative stress in CKD patients [1].

The gut microbiota plays an important role in regulating many aspects of immunity, protecting the host against pathogenic microbes and producing vitamins and other essential nutrients. The density of bacteria in the gastrointestinal tract is $\sim 10^{13}$–10^{14} cells/g of faecal matter, and the colon possesses 70% of the total GI tract bacteria [1, 2]. Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia are the five bacterial phyla present in the human gut and two of these, Bacteroidetes (Bacteroides, Prevotella and Xylanibacter) and Firmicutes (Ruminococcus, Clostridium, Lactobacillus, Eubacterium, Faecalibacterium and Roseburia) dominate the flora [3]. The gene content of the total DNA of this bacterial mass is greater than 4 million genes, whereas our genome is only composed by 23 000 genes. The cell number of this internal organism is 100 times greater than the total cell number of the human body and it has recently been shown that richness of this gut flora is associated with better metabolic profile in human [2].

Few studies have documented the composition of gut microbiota during CKD [4–8], and to date, there is not enough published data to confirm if CKD patients present an altered gut microbiota composition. In 1996, Hida *et al.*, using traditional plating methods for the analysis of faecal samples did not find significant differences in the total number of colic bacteria in haemodialysis (HD) patients versus healthy individuals. In addition, in HD patients, these authors found an increased number of intestinal aerobic bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus*) and intestinal anaerobic *Clostridium perfringens* as well as a decreased number of intestinal anaerobic bacteria (*Bifidobacterium* species) [4]. Wang *et al.* [5] were the first to analyse the gut microbiota by real-time PCR and found that *Bifidobacterium* species, *B. catenulatum*, *B. longum*, *B. bifidum*, *Lactobacillus plantarum* and *Lactobacillus paracasei* were detected at lower rates in peritoneal dialysis patients when compared with healthy individuals. Vaziri *et al.* [6], using microarray analysis, observed significant differences in the abundance of 190 microbial operational taxonomic units (OTU) between healthy individuals and haemodialysis patients, who presented an increased abundance of *Enterobacteriaceae*, particularly the OTUs containing certain clusters of *E. coli* sequences. A recent study supported the hypothesis that

CKD patients may present an expansion of bacterial families possessing urease, uricase, and indole- and p-cresol-forming enzymes and a reduction of bacterial families possessing butyrate-forming enzymes, which contribute to the uraemic toxicity and systemic inflammation in these patients [7].

A recent study from our group showed that the average number of bands evaluated by denaturing gradient gel electrophoresis (DGGE) was not different between healthy individuals and non-dialysis CKD patients. However, the sequencing of PCR products from representative bands showed *Listeria monocytogenes* and *Flavobacteriaceae bacterium* in patients, and uncultured *Lachnospiraceae bacterium* and *Butyrivibrio crossotus* in healthy individuals [8]. More research is needed to evaluate the gut microbiota profile in CKD patients and, with the advent of high-throughput sequencing techniques like pyrosequencing, a technology that provides rapid, short-read sequencing of bases, studies will be able to better identify the bacterial phylotypes of these patients.

There are a limited number of studies on the effects of gut microbiota composition on inflammation during CKD. Recently, Shi *et al.* [9] showed that bacteria detected in the blood were also distributed in the gut of CKD patients and the bacterial DNA concentration was positively correlated with plasma levels of C-reactive protein and interleukin-6. Studies have been more focused on the effects of uraemic toxin production by gut microbiota and its impact on inflammation [10]. In fact, a high urea load can be delivered to the intestine from the plasma of uraemic patients. Then, urease, expressed by some gut bacteria species, may promote urea hydrolysis leading to the formation of large amounts of ammonia further converted to ammonium hydroxide. Both ammonia and ammonium hydroxide can alter the intestinal epithelial tight junctions and promote the entrance of lipopolysaccharides (LPS) and uraemic toxins into systemic circulation [6].

Therefore the imbalance in gut microbiota associated with alterations in colonic epithelium contributes to the accumulation of gut-derived uraemic toxins. Toxic gases, indoxyl sulphate, p-cresyl sulphate, amines, ammonia and trimethylamine n-oxide (TMAO) as well as precursors for lipopolysaccharides (LPS) may be absorbed into the bloodstream and be responsible for systemic inflammation [1, 3]. These compounds are cytotoxic and will induce cardiovascular and immune alterations. Indeed, several studies have shown that these toxins are reliable markers of cardiovascular disease and mortality in CKD patients [11, 12]. One of the mechanism by which uraemic by-products may induce toxicity has been recently clarified. These toxins stimulate the production of reactive oxygen species (ROS) through a pathway that likely involves NADPH oxidase (membrane-bound enzyme complex that generate superoxides). This ROS production triggers the mitogen-activated protein kinase (MAPK)/NF-κB (nuclear factor κB) pathway that results in the production of proinflammatory cytokines, chemokines and adhesion molecules [13, 14]. Furthermore, LPS is detected by toll-like receptor 4 (TLR4) on endothelial cells and monocytes/macrophages, leading to the activation of NF-κB and AP-1 (activator protein-1) [15].

Vanholder and Glorieux recently described the role of gut microbiota in the production of uraemic toxins and the subsequent modification of intestinal physiology in CKD patients. They discussed the therapeutic options that could help modify the negative effects (including inflammation) provoked by this gut microbiota imbalance [10]. According to these authors, studies in CKD patients using these therapeutic strategies are scarce, and mostly not randomized, or if randomized, results are often inconsistent. In addition, no study has evaluated the effects of interventions on gut microbiota profile.

A recent review hypothesized that a restriction in some amino acids through a low-protein diet could be an interesting strategy to reduce uraemic toxins in CKD patients on a conservative treatment [16]. The probiotic use may be effective to minimize inflammation and oxidative stress in CKD patients [1, 3]. Ranganathan *et al.* [17] after a probiotic supplementation in non-dialysed CKD patients, observed an improvement in quality of life and a reduction in serum uric acid and creatinine levels. In contrast, Hyun *et al.* [18] showed that there was no significant effect of probiotics on the reduction of uraemic toxins in paediatric dialysis patients.

The effectiveness of the use of probiotics in the treatment of inflammation in CKD patients has not been extensively examined. Recently, Wang *et al.* [19] reported a reduction on serum levels of pro-inflammatory cytokines in peritoneal dialysis patients after 6 months of probiotic supplementation. However, several confounding factors and errors in probiotics studies can hide their deleterious effects [20].

Studies with prebiotics are scarce. Meijers *et al.* [21] observed that a 4-week prebiotic oligofructose-inulin supplementation significantly reduced p-cresyl sulphate levels in haemodialysis patients. Recently, Vaziri *et al.* [22] showed that a high resistant starch diet as prebiotic source retards CKD progression and attenuates oxidative stress and inflammation in CKD rats.

Where do we stand now? The basic mechanism of the gut-kidney crosstalk must be first clarified. Then, we need more studies to evaluate the possible therapeutic strategies able to modulate the gut microbiota and reduce uraemic toxins, as well as the efficacy to alleviate the inflammation process in CKD patients. This promising field of research will certainly lead to the discovery of new therapeutic strategies for regulating the gut microbiota and reducing inflammation in CKD patients.

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(See related article by Vanholder and Glorieux. The intestine and the kidneys: a bad marriage can be hazardous. *Clin Kidney J* (2015) 8: 168–179.)

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