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ABSTRACT
Gut microbiota can be considered a real organ coordinating health and wellness of our body. It is made of more than 100 trillions of microorganisms, thus about 3 times higher than the number of human body cells and more than 150 times than human genes containing 1000 different microbe species.

It has been described a symbiotic relationship between gut and kidney, confirmed by several observations. This is a bi-directional relation with a mutual influence, even when kidney disease occurs, and consequent alterations of intestinal microbiota and production of uremic toxins, that in turn worsens kidney disease and its progression.

Our review analyzes the components of gut-kidney axis and relative clinical consequences.

KEYWORDS: urea, microbioma, indoxyl sulphate, p-cresol sulphate, Mediterranean Diet, VLPD, Short Chain Fat Acid, prebiotics, probiotics
INTRODUCTION

Already in 1963 Giordano and in 1964 Giovannetti (1) realized that urea was not only a marker of reduced renal function, but a real uremic toxin. They understood how to reduce serum urea in subjects with chronic kidney disease without inducing malnutrition but improving symptoms related to hyperazotemia (1). Later, urea was put aside as marker of renal function, then was used, through Kt/V (2), to measure dialysis efficiency and protein intake (3).

Nowadays, we know that chronic kidney disease is characterized by a progressive increase of waste products with different molecular weights that may alter cellular functions (here comes the name “uremic toxins”). Moreover, it is well known the importance and the contribution to cardiovascular disease in chronic kidney disease (4). Very recently, urea has been reconsidered as uremic toxin (5). In fact, it is now known the relation between urea and cyanate production and its role in CKD through post-translational modification of proteins (6), and the action exerted by urea degradation products on the intestine and the integrity of intestinal barrier (7).

Intestinal microbiota is a real organ that coordinates in a flexible way health and wellness of our body (8): it is made of more than 100 trillions of microorganisms, thus about 3 times higher than the number of human body cells and more than 150 times than human genes containing 1000 different microbe species. Definitively, it has the same metabolic potential of liver (8). Intestinal microbiota may be considered as an external milieu with an estimated weight of about 2 kilos, lasting the entire life and interacting with all body organs, with regulation actions on immune system, but also with a potential in production of toxins (9-15). In fact, in the entire intestinal tract there is physiologically a balance between saccharolytic and proteolytic fermentation and an alteration of this balance may cause detrimental effects in chronic kidney disease or in dialysis patients (16).

Nowadays, there is great interest on this topic with more than 25000 papers published in PubMed (Figure 1), more than 10000 of them only in the last two years.
**Urea and Intestine**

Chronic kidney disease patient is notoriously a subject with micro-inflammation and oxidative stress, that may be caused by several factors (17). Recent evidence shows that high levels of urea contribute to deteriorate these aspects, themselves damaging permeability of intestinal barrier (5). In fact, urea easily spread in the intestinal fluid where it is degraded by bacterial urease enzymes, then it is hydrolyzed in ammonium hydroxyde that increases fecal pH with a consequent alteration of intestinal cellular junctions (5,18). In addition, high levels of urea cause change of intestinal microbiota promoting proteolysis (19) with production and absorption of uremic toxins, such as indoxyl sulfate and p-cresol sulfate (20).

Very recently, Andersen and coll showed in mouse experiments that disbiosis caused by chronic kidney disease-related inflammation produced a diffusion through intercellular spaces of intestinal bacteria to the liver, with an increased release of endotoxins (21). In fact, the authors showed increased levels of serum pentraxin-2/serum amyloid-P (the equivalent of human Pentraxin-1/C-reactive protein) in mouse with disbiosis (21).

A recent systematic review form Vanholder and coll evidenced that indoxyl sulfate damaged endothelium, hepatocytes, muscle cells, myocytes, renal proximal tubular cells, intestinal cells, so as p-cresol sulfate damaged leucocytes, adipocytes, renal proximal tubular cells, intestinal cells and myocytes. As a consequence of high values of indoxyl sulfate and p-cresol sulfate, relevant pathophysilogic changes occurred such as production of reactive oxygen species, interaction between leukocytes and endothelium, increase of cellular proliferation and aging, myocytes hypertrophy, cardiac fibrosis, inflammation, oxidative stress, cytokines production, inflammatory genes expression, RAAS activation, renal tubular damage, insulin-resistance, lipogenesis reduction and lipolysis activation (22).

Intestinal alterations in chronic kidney disease may be a consequence of a erroneous nutritional therapy with the use of a low content of fibers due to the fear of hyperkalemia. Hence, it is necessary to delete some ambiguities and misleading beliefs: dietetic administration of vegetal fibers and fruit do not cause serious hyperkalemia because the simultaneous consumption of alkali (with vegetables and fruit) induces intracellular potassium shift (22); in fact, hyperkalemia in chronic kidney disease patients with an efficient urine output especially occurs when RAAS inhibitors and aldosterone antagonists are prescribed (23). Furthermore, a lower intake of fibers causes a reduction of short-chain fatty acids (SCFAs) into intestinal lumen, as propionic and butyric acids. SCFAs derive from bacterial fermentation and are normally present at high concentrations into the intestin. These metabolites represent a junction between microbiota and immune system (24-25). After internalization into enterocytes to be used as energy fuel, they increase expression of antimicrobial peptides secreted on the external surface of intestinal cells (26, 27), and modulate immune mediators production, as IL-18, a cytokine fundamental in reparation and conservation of cellular integrity, and other cytokines and chemokines (26,27). Moreover, SCFAs regulate differentiation, recruitment and activation of immune system cells like neutrophils, macrophages and lymphocytes T(26, 27).

The use of a nutritional therapy with a very low protein content and a high quantity of vegetables and fruit with the supplementation of essential amino acids and ketoanalogues of non essential aminoacids ensures the lowering of urea levels with an appropriate amount of fibers, promoting the building of a physiological intestinal microbioma (23, 28-30).

**Microbiote and Kidney**

Symbiotic gut microbiota has the important function of preserve the intestinal barrier through
mucus, antimicrobial peptides and IgA production that contribute to maintain microbiota into the intestinal lumen and far from epithelial intestinal cells. Intestinal immune system is very tolerant with symbiotes, so that epithelial cells are able to recognize microbes through recognition receptors like Toll-Like Receptor 4 (31). Intestinal response to inflammation and infections is very complex and depends from collaborations with symbiotic bacteria and from regulatory mechanisms including T-helper cells 1 and 2, MYD88 that induces down-regulation of IL-1 receptor-associated kinase 1 (IRAK1) with the consequent activation of NK-kB cascade and production of antimicrobial proteins and pro-inflammatory cytokines (32).

The exposure of intestinal cells to lipopolysaccharides induce them to the secretion of TGF-b, B-cell-activating factor of TNF family (BAFF), and a ligand inducing proliferation (33). As a consequence, microbiota immune cells (dendritic CD103 cells, T-cells secreting IL-10 and TGF-b) activate their tolerance responses and stimulate specific intestinal IgA (30).

A subclinical endotoxemia is a potential cause of inflammation in chronic kidney disease. An altered immune response and production of pro-inflammatory cytokines at the intestine level may accelerate progression of renal disease and cardiovascular complications (31,34–37).

Microbiote and Aging

Aging is a physiological senescence process of body functions with age; several studies evidenced a tight relation between age and microbiota (38). Already during intrauterine period intestine is sterile and it has been observed a different bacterial colonization depending on vaginal or cesarean delivery, with a higher prevalence of Clostridium difficilis after cesarean delivery compared with a higher prevalence of Bifidobacteria, Proponiumbacteria and other symbiontes after vaginal delivery (38–41). This different colonization has remarkable influence on intestinal microbiota building in the adult age (42), and on the onset of metabolic disorders like type 2 diabetes, obesity, atherosclerosis, gastrointestinal inflammatory diseases (43,44). Finally, the transition from the adult age to the elderly induces a rapid change of microbiota with a reduction of Firmicutes and an increase of Bacteroidetes (45,46).

Throughout life, diet considerably influences intestinal microbiota composition inducing a shift from physiological saccharolytic bacteria to proteolytic bacteria (15, 16).

The influence of gut and microbiota composition on chronic kidney disease has already been issued from the scientific literature (31, 37, 47). On the other side, the aspect not previously explored is whether low-protein diet may influence the quality and composition of intestinal microbiota. Moreover, aging seems to preponderantly alter microbiota senescence so as kidney deterioration (48–50).

Mediterranean Diet and Disbiosis

Healthy effects of Mediterranean diet on cardiovascular complications (51,55) and neoplasia incidence (56,57) are known all over the world. Mediterranean diet is characterized by the assumption of fresh products, and great amount of vegetables and fruit with use of legumes, nuts, olive oil, fish and a moderate consumption of red wine.

Knowledge of a relationship between microbiota and chronic kidney disease has led nephrologists to study with even greater interest the relation between Mediterranean diet and chronic kidney disease. Mediterranean diet efficiently contributes to intestinal microbiota building; in fact, it
promotes intestinal development of saccharolytic bacteria with a competitive reduction of proteolytic bacteria that instead induce p-cresol and indoxyl sulfate production (58). Several researchers confirmed these data and believe that a physiological microbiota may delay progression of renal disease (59,60) and mortality in chronic kidney disease patients (61,62).

**Short Chain Fat Acid (SCFA) and Disbiosis**

We have previously described the role of intestinal microbiota in the production of SCFAs from fibers metabolism and in the immune-regulatory action at the intestine level (24-27). SCFAs are produced in the colon and distal small intestine by anaerobic bacteria following fermentation of complex carbohydrates. The major compounds are acetic acid, butyric acid and propionic acid, with positive effects on microbiota and intestinal mucosa. It is known that they exert anti-inflammatory, anti-cancer, antibacterial and antidiabetic effects. Lower values with a consequent dysbiotic gut contribute to the pathogenesis of different diseases such colitis, type 2 diabetes, rheumatoid disease and multiple sclerosis (63). Synthesis SCFAs may also be administered orally (63). Supplementation of SCFA has been shown to have anti-inflammatory actions both in intestinal epithelial cells (64) and in the cardiovascular system (64). They also positively influence auto-immune diseases (64-70).

**Urea, Disbiosis and Outcomes**

In chronic kidney disease and in dialysis patients there is an accumulation of uremic toxins with an intermediate molecular weight such as phenylacetylglutamine, hippurate, indoxyl sulfate and p-cresol sulfate (22). These are protein-bound solutes with a scarce dialytic clearance (36% for indoxyl sulfate and 31% for p-cresol sulfate, respectively). Several studies described the toxicity of indoxyl sulfate and p-cresol sulfate on kidney disease progression and cardiovascular system (4,22,71-80). Our group was one of the first to evidence the efficacy of a very-low protein-diet supplemented with ketoanalogues and essential amino-acids and with a high fibers content in reducing of 35% indoxyl sulfate levels (81); this effect was studied later (82). Preliminary data from MEDIKA study (“Renal Effects of Mediterranean Diet and Very Low-protein Diet With Ketoacids (VLPD) on Physiological Intestinal Microbiota in CKD” registered in ClinicalTrial.gov with the number NCT02302287, ongoing) show that in 30 patients a very-low protein diet significantly reduces indoxyl sulfate levels of 72% (from 0.46±0.12 to 0.13±0.05 mcg/mL, p=0.002) and il p-cresol sulfate levels of 51 % (p<0001) (Figure 2) (83). In detail, VLPD is a vegetarian diet with no animal proteins and 0,3 g/kg of body weight/day of vegetable proteins with supplementation of essential amino-acids and ketoanalogues of non essential amino-acids, and a caloric intake of 30-35 kcal/kg of body weight/day (84).
Moreover, nowadays it has been put back at the top the idea that urea is a toxin and it must be therefore treated. Regarding intestinal microbiota, disbiosis in chronic kidney disease is determined also by high levels of urea that cause enterocolites (not clinically relevant) through ammonium hydroxide formation from urea decomposition and ammonium ion hydroxilation due to intestinal bacterial urease. On the other side, high levels of urea also cause an exaggerated production of cyanate with consequent protein carbamylation and atherosclerotic effects (5). Therefore, urea, an old and forgotten molecule, must be reconsidered now as a real uremic toxin.

VLPD is able to efficiently reduce urea levels in patients with chronic kidney disease, leading its values into the normal range in many patients despite a reduced residual renal function lower than 15 ml/min (29, 84, 85). A prospective randomized cross-over controlled trial showed that urea reduction in 60 subjects induced a significant cyanate lowering (86); also correlation between urea and homocitrulline was significant ($y = 10.2 x + 6.97$, $r = 0.72$; $p<0.001$) (Figure 3). Figure 4 shows that VLPD was more efficient than Mediterranean diet in reducing urea and therefore cyanate levels (86). Urea is directly involved in the pathogenesis of cardiovascular diseases in chronic kidney disease patients through the generation of isocyanic acid and protein carbamylation, both processes with a high atherosclerotic impact (5, 87-89).

Moreover, VLPD allows a better control of metabolic acidosis in chronic kidney disease because it ensures bicarbonate administration with vegetable proteins (90).
Microbioma and Pre/Pro-Biotics

Probiotics (microorganisms belonging to human microbiota) and prebiotics (digestible but not fermentable oligosaccharides) are useful to restore intestinal microbiota and promote health of the host also in chronic kidney disease subjects (91). Prebiotics have synergistic action with probiotics and administered together (symbiotics) may have an important role in restoring the physiological intestinal microbiota and delaying chronic kidney disease progression (92, 93).

Scientific literature described the relationship between use of symbiotics and reduction of indoxyl sulfate and p-cresol sulphate (94–96). This effect is evident also in patients already in hemodialysis treatment (97). In fact, the use of SCFAs to restore physiological levels of anaerobic bacteria (Sutterellaceae, Lactobacillaceae and Bacteroidaceae) and not of aerobic bacteria such as Finucutes...
Proteobacteriae and Actinobacteriae, that are at higher concentration in the intestine of chronic kidney disease patients, allows the reduction of uremic toxins like indoxyl sulfate and p-cresol sulfate (98).

Very recently, Soleimani has shown, (with a prospective randomized, double-bin, placebo-control clinical trial) that supplementation for 12 weeks of pro-biotics produced in 60 diabetic hemodialysis the reduction of 22 mg/dl for glucose, 0.4% for HbA1c, 6.4 mcU/ml for serum insulin, 1933 ng/ml for the C-reactive protein compared to the placebo group, and then, in conclusion, improvement in diabetic parameters (99).

Moreover, use of natural symbiotics like beta glucan contained in whole-grain pasta induces increase of SCFAs such as 2-methyl-propanoic, acetic, butyric, and propionic acids and significant reduction of indoxyl sulfate and p-cresol sulfate (100,101).

Conclusions

Researchers investigated a lot in the last years the interrelation between kidney and gut, the so-called gut-kidney axis, and between intestinal microbiota or disbiosis and kidney damage.

It is indisputable that inflammation and intestinal disbiosis influence chronic kidney disease progression and, conversely, chronic kidney disease influences microbiota changes towards a proteolytic flora instead of a saccharolytic one with production and absorption of uremic toxins such as indoxyl sulfate and p-cresol sulfate. Therefore, taking into consideration the mutual influence between kidney and intestine, the care of intestinal disbiosis must be a therapeutic target in chronic kidney disease (102).

Nowadays, the role of these uremic toxins in promoting negative cardiovascular outcomes in chronic kidney disease patients it is well known in the scientific community. Also, it is well recognized the relationship between indoxyl sulfate and cardio-renal syndrome through mechanisms favoring oxidative stress, production of reactive oxygen species, nicotinamide-adenine-dinucleotide-phosphate activity, and reduction of glutathione levels (103, 104). Similarly, the role of intestinal microbiota on nitrose cycle, that induces reduction of nitric oxide in chronic kidney disease through reduction of intestinal species that are able to transform ammonium in nitrate was already explored (105).

Use of a proper nutrition, as Mediterranean diet is, may induce the development of intestinal bacteria flora able to control the intestinal cellular immune system, contrast the formation of uremic toxins in the intestine, and favor production of SCFAs.

Moreover, chronic kidney disease influences microbiota characteristics especially through high levels of urea, that is reconsidered a real uremic toxin that need therefore to be treated (5). Only the use of a low-protein content nutrition, such as VLPD with supplement of essential amino-acids and ketoanalogues, allows an efficient control of urea plasma levels. In fact, administration of ketoanalogues, permits recycling urea nitrogen to transform the ketoanalogous in the corresponding no-essential amino acid, maintaining a proper dose of energy intake. In conclusion, we think that the utilization of nutritional therapy to reduce urea levels and restore physiological microbiota in chronic kidney disease is mandatory.
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