Uremic Solutes Produced by Colon Microbes

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Key Words

Uremia · Microbiome · Colon

Abstract

Background: Colon microbes produce a large number of organic compounds that are foreign to mammalian cell metabolism. **Summary:** Some of the compounds made by microbes are absorbed in the colon and then normally excreted by the kidneys. Accumulation of these compounds in the plasma as uremic solutes may contribute to illness in patients whose kidneys have failed. Mass spectrometry is expanding our knowledge of the chemical identity of the colon-derived uremic solutes, and DNA sequencing technologies are providing new knowledge of the microbes and metabolic pathways by which they are made. Because they are made in an isolated compartment by microbes, their production may prove simpler to suppress than the production of other uremic solutes. **Key Messages:** To the extent that they are toxic, suppressing their production could improve the health of renal failure patients without the need for more intensive or prolonged dialysis.

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The Colon Microbiome

The human gut harbors over 100 trillion microbes that comprise a symbiotic ecosystem [1, 2]. This colon ‘microbiome’ performs multiple functions which have not been

fully elucidated. Its most important function through evolutionary history has probably been to degrade plant polysaccharides that cannot be digested in the small intestine, providing energy to the human host in the form of short chain fatty acids. While this process provides only a small portion of the energy used daily in humans consuming an ‘industrialized’ diet, it can be a crucial source of energy when the diet contains more unprocessed plant foods [3]. Colon microbes also produce micronutrients including some vitamins and can produce hormones that promote fat storage. In addition, the colon microbiome limits colonization of the gut by pathogens and stimulates the human immune system in useful ways.

Uremic Solutes Produced by Colon Microbes

In addition to its beneficial products, the colon microbiome produces a large number of solutes that have no biologic value. Many of these solutes are absorbed through the colon epithelium and then normally excreted by the kidneys, often after conjugation. The colon-derived solutes that have been most extensively investigated are indoxyl sulfate and p-cresol sulfate, which are formed as depicted in figure 1. These compounds have received the most attention because they are formed in relatively large quantities. They were therefore detected in human urine by the methods of classic organic chemistry and then shown to accumulate in the plasma when the kidneys fail.

Fig. 1. Microbial generation of indoxyl sulfate from tryptophan and of p-cresol sulfate from tyrosine. Tryptophan is converted to indole by tryptophanase, which is found only in microbes. Absorption of indole in the colon is then followed by oxidation and sulfation in the liver. Tyrosine is converted by microbes in two steps to 4-hydroxy phenylacetic acid, which is then decarboxylated to p-cresol by an enzyme which has been shown to be present most

notably in *Clostridium difficile*. Sulfation of p-cresol is then accomplished in the colonic epithelium and possibly also in the liver. Older reports measured p-cresol rather than p-cresol sulfate in the plasma of dialysis patients because assay techniques employed acidification which hydrolyzed the conjugate. Lesser portions of both indoxyl and p-cresol are conjugated with glucuronic acid rather than sulfuric acid.

As described below, there is considerable though not conclusive evidence that they are toxic.

Mass spectrometry has made it possible to identify many more uremic solutes and to determine which of these are made by colon microbes. Kikuchi et al. [4] first used mass spectrometry to detect solutes that accumulate in the plasma of rats with renal insufficiency. Sato et al. [5] subsequently demonstrated the capacity of mass spectrometry to detect compounds which accumulate in the plasma of hemodialysis patients. Lists of known uremic solutes have been prepared by combining mass spectrometric findings with results obtained using other analytic methods [6–9]. We recently identified an additional 48 uremic solutes by comparing plasma samples from hemodialysis patients and normal subjects using an established metabolomic platform [10]. Combination of these solutes with those identified in previous reports yielded a list of more than 270 known uremic solutes.

Solutes produced by colon microbes can be identified by combining mass spectrometry with maneuvers that

suppress microbial solute production. A pioneering study by Wikoff et al. [11] identified solutes produced by gut microbes by comparing plasma from germ-free rats and conventional animals. Of note, some solutes shown to be of microbial origin were later found to accumulate in the plasma of mice lacking the anion transporter OAT1, suggesting that they are removed from the body largely by renal tubular secretion [12]. Kikuchi et al. [13] subsequently identified solutes whose plasma levels were reduced by the administration of the oral sorbent AST-120 in rats with renal insufficiency. Aronov et al. [14] initially identified colon-derived uremic solutes by comparing plasma solute profiles in dialysis patients with and without colons. We recently identified six additional colon-derived solutes by reanalyzing the samples collected by Aronov et al. [14], using an established metabolomic platform [10]. Interestingly, these compounds were all phenyl and indole compounds. Query of the BioCyc database identified potential microbial sources for these colon-derived solutes. Com-

Table 1. Colon-derived uremic solutes

Solute	References
Indoxyl sulfate	[11, 13, 14]
Indoxyl glucuronide	[14]
5-Hydroxyindole	[14]
3-Indolepropionic acid	[11, 14]
p-Cresol sulfate	[11, 13, 14]
p-Cresol glucuronide	[43]
Phenol sulphate	[11, 13]
Phenol glucuronide	[14]
Alpha-N-phenylacetyl-L-glutamine	[14]
Phenylpropionylglycine	[11]
Cinnamoylglycine	[11, 14]
4-Ethylphenyl sulfate	[13]
Hippuric acid	[11, 13, 14]
Phenylacetic acid	[10]
2-Aminophenol sulphate	[10]
3-Hydroxyhippuric acid	[10]
2-Methoxyphenol sulfate	[10]
4-Methylcatechol sulfate	[10]
3-(3-(Sulfoxy)phenyl) propanoic acid	[10]
Trimethylamine N-oxide	[24, 25]

These compounds accumulate in the plasma in renal failure, and evidence in the references suggests that they are derived from colon microbial metabolism.

binning the results of these reports yields a total of 20 colon-derived uremic solutes as listed in table 1. This list will undoubtedly get longer as more mass spectrometric studies are performed.

The Toxicity of Colon-Derived Uremic Solutes

Evidence has accumulated that the best known colon-derived uremic solutes, p-cresol sulfate and indoxyl sulfate, are toxic [15–21]. Recent studies have concentrated on the possibility that these compounds cause vascular injury. High levels of p-cresol sulfate have been correlated with cardiovascular diseases and mortality in patients with chronic kidney disease (CKD) [18, 19]. Indoxyl sulfate has been shown to induce endothelial dysfunction via an induction of oxidative stress in vitro [20] and high serum levels have been associated with cardiovascular disease and mortality in CKD [21]. The structural relation of the uremic indoles and phenols to neurotransmitters has also encouraged speculation that they impair cognitive function, and it has recently been noted that they could do this by interfering with cleaning of the brain interstitium by the blood-brain barrier.

Another colon-derived solute, trimethylamine N-oxide (TMAO), has recently attracted considerable attention as a risk factor for cardiovascular disease [22]. TMAO is not made from amino acids but rather from choline, which is in turn derived largely from dietary meat. Initial studies associated high TMAO levels with increased risk of cardiovascular events in people who did not have renal failure [23, 24]. Some studies have since associated high TMAO levels with cardiovascular disease in patients with renal failure, but this has not been a uniform finding [25, 26].

The Colon Microbiome May Be Altered in CKD

Just as mass spectrometry has increased our knowledge of uremic solutes, DNA sequencing technology has greatly increased our knowledge of the diversity and function of the colon microbes [27]. Application of this technology has recently revealed qualitative and quantitative changes in the gut microbiome in CKD [28]. Vaziri et al. [29] first assessed the taxonomy of the colon microbiome in hemodialysis patients using the Affymetrix Phylochip. Significant differences were found in the prevalence of a number of specific bacterial types. Because the study was conducted with an early version of the Phylochip it was not possible to assess microbial diversity. However a companion study using a later chip identified reduced diversity of the colon microbiome in rats with renal insufficiency.

Several factors could be responsible for alteration of the colon microbiome in patients with renal insufficiency. The first, particularly among patients with end-stage renal disease, is alteration of the diet. End-stage renal disease patients also frequently receive antibiotics and have prolonged colon transit time. In addition, the colon microbiome is itself exposed to increased concentrations of various compounds that accumulate in renal failure and can be metabolized by microbes. The most prominent of these is urea, which is converted to ammonia by microbial urease [30, 31]. Other examples are creatinine, uric acid, and oxalate which enter the colon in increased quantities in renal failure and are degraded by microbes [32–34].

Alterations in the microbiome in CKD have implications for future therapy, as described below. But much more needs to be learned about the microbiome before we can manipulate it to suppress solute production. Remarkably, microbial populations with divergent taxonomic profiles can have similar functional capacity as

reflected by the presence of genes encoding enzymes for various metabolic pathways [35]. Functional capacity as well as taxonomy must therefore be assessed to gauge the extent to which disease alters the microbiome and to identify microbial processes that may be over or under represented in disease sufferers. One method of assessing functional capacity is to predict the genetic makeup of the microbiome based on the results of 16S rRNA gene profiling and the genetic makeup of microbial species for which whole genome sequences have been reported. This approach, which limits cost, provided a remarkably accurate overall picture of microbial genes in samples from the Human Microbiome Project [36]. It has limited ability, however, to assess the prevalence of unusual microbial genes which are expressed irregularly in closely related species. The prevalence of such genes can be more reliably assessed by 'shotgun' sequencing of the microbial DNA present in fecal samples, although compared to the 16S rRNA-based metagenomic inference method this has the disadvantage of missing genes from low abundance species [37, 38]. Further studies with both of these complementary methods will therefore be needed to assess the prevalence in the microbiome of genes encoding enzymes responsible for the production of important uremic solutes.

Reducing the Production of Colon-Derived Uremic Solutes

Treatment of renal failure is currently focused on removing solutes by dialysis and largely ignores the alternate strategy of suppressing solute production. Maneuvers to suppress production, however, could prove particularly effective for solutes that are made in an isolated body compartment by microbes. Plasma levels of these solutes vary widely among patients with the same degree of renal insufficiency, further suggesting that solute production is variable and should be susceptible to manipulation. One potential means to limit the production of colon-derived solutes is to alter the food supplied to colon microbes. On an 'industrialized' diet, the colon microbes receive up to 10 g of amino acids in the form of incompletely digested proteins, sloughed intestinal cells, and secretions. The quantity of amino acids delivered to the colon may be increased in renal failure due to impaired digestion of proteins in the small intestine. These amino acids are the source of p-cresol sulfate, indoxyl sulfate, and some other uremic solutes produced by colon microbes.

Dietary protein restriction can limit delivery of amino acids to the colon but may have adverse effects and, if severe, result in negative nitrogen balance. An alternate means to reduce the microbial production of waste solutes from amino acids is to increase fiber intake. The term fiber includes carbohydrates and related compounds that escape digestion in the small intestine. With high-fiber intake these compounds provide energy for microbial growth. Amino acids reaching the colon are then used for synthesis of microbial proteins rather than being converted into waste solutes. Recent studies have shown that increasing fiber intake can reduce the plasma concentrations of p-cresol sulfate and indoxyl sulfate in hemodialysis patients [39, 40]. Other studies have shown that among people with normal kidney function, those consuming a vegetarian diet which is high in fiber produce less p-cresol sulfate and indoxyl sulfate than those consuming an unrestricted diet [41].

The studies cited above show the increasing fiber intake can reduce production of p-cresol sulfate and indoxyl sulfate, which are made from the amino acids tryptophan and phenylalanine/tyrosine, respectively. Not all the colon-derived uremic solutes are made from amino acids, however. We have recently found that many colon-derived solutes are derived rather from polyphenols and related compounds in plant foods [10]. Plants contain thousands of such compounds with an extraordinary variety of structures. Many of them escape digestion in the small intestine and are metabolized by colon microbes. Several grams per day of dietary polyphenols are thereby transformed into new substances which are normally excreted by the kidney, often after conjugation in the liver. At present we do not know whether any of these substances are toxic. And consideration of the health effects of plant polyphenols has been focused largely on their potential benefit with particular attention to their potential antioxidant and anticarcinogenic activities. Accumulation of metabolites which are normally excreted by the kidney, however, could turn these putative benefits to harm in patients with renal failure.

Another potential means to reduce the burden of colon-derived solutes is to administer sorbents that bind their precursor compounds in the colon. The carbon-based sorbent AST-120 reduces plasma levels of indoxyl sulfate and can also reduce levels of p-cresol sulfate and other solutes [42]. Further work is required to determine whether use of sorbents can provide long-term health benefits in patients with renal failure.

Perhaps the most promising potential means to reduce microbial production of toxic solutes is to modify the

composition of the microbiome. Limited studies have so far attempted to alter the microbiome by administration of 'probiotic' bacterial strains which do not produce toxins. A major problem is survival in the microbiome of the probiotic bacteria. Knowledge of the genetic makeup of the microbiome may ultimately provide more effective means to adjust its composition. Hopefully, it will be possible to introduce microbes in which the capacity to produce toxins has been genetically deleted but which are otherwise well fitted for survival in the colon microbial ecosystem.

Increasing the Removal of Colon-Derived Solutes

The alternative to suppressing solute production is to improve solute removal by dialysis. Indoxyl sulfate and p-cresol sulfate, the two best known colon-derived solutes, are more than 90% bound to plasma albumin. Because only the portions of bound solutes are available for diffusion through the dialysis membrane, their clearances are only a small fraction of the urea clearance during conventional dialysis. The clearance of the bound solutes, however, can be greatly increased by increasing the dialysate flow and dialyzer size. The clearance of bound solutes can also be increased by combining high-volume ultrafiltration with hemodialysis and adding sorbents to the dialysate. Theoretically, sustained application of these techniques could reduce the plasma concentrations of bound

solutes without increasing either the duration or frequency of dialysis. More extensive clinical studies are required to determine the extent to which this can be accomplished in practice.

Conclusion

Colon microbes produce numerous compounds which are normally excreted in the urine and accumulate in the plasma when the kidneys fail. While there is evidence that some of the colon-derived compounds are toxic, our knowledge of their toxicity is far from complete. Better knowledge of solute toxicity could lead to new means to reduce the levels of toxic solutes in renal failure patients. In particular, because the colon-derived solutes are made in an isolated compartment by microbes, it may prove simpler to reduce their levels by suppressing their production than by enhancing their removal.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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