Review

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Urolithins and intestinal health

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SUMMARY There are trillions of microorganisms in the human intestine. They can react to the intestinal micro-environment by metabolizing food or producing small molecular compounds to affect the host's digestive ability and resist the risk of infection and autoimmune diseases. Many studies have revealed that intestinal flora and its metabolites play an important role in human physiology and the development of diseases. Urolithins are kind of intestinal microbiota metabolites of ellagitannins (ETs) and ellagic acid (EA) with potent biological activity *in vivo*. However, different individuals have different intestinal flora. According to the different metabolites from ETs and EA, it is divided into three metabo-types including UM-A, UM-B and UM-0. This paper reviews the origin of urolithins, the urolithin producing microorganisms and the effects of urolithins on regulating intestinal diseases. This review will provide a theoretical basis for the regulation of urolithins in the homeostasis of intestinal flora and a reference for the scientific utilization of urolithins and foods rich in ETs and EA.

Keywords Urolithin, intestinal microbiota, intestinal health

1. Introduction

It is estimated that the number of bacteria in human intestine is about 10 times that of human cells. The micro-ecological balance of intestinal flora affects energy absorption and immune system, thus affecting human health. Studies have reported that small molecular compounds produced by intestinal microbiota have direct correlation with human health. For example, short chain fatty acids (SCFAs), produced by *Bacteroides* and *Firmicutes* through anaerobic fermentation of dietary fiber, can enhance host immunity (*1,2*). Indole produced by intestinal flora can regulate the host's immune system and inflammatory diseases (*3*). Flavonoid metabolites of intestinal flora can reduce the prevalence of obesity (*4*). Meanwhile, specific intestinal bacteria and their products can lead to human diseases. For example, the small molecule TMAO produced by intestinal microbiota from nutrients rich in choline is related to many metabolic diseases (*5,6*) Colibactin produced by a polyketide synthase positive (PKS^+) *Escherichia coli* can promote the occurrence of colorectal cancer (CRC) (*7*). Therefore, it is of great significance to study the mechanism of the intestinal microbiota metabolites and their occurrence together with their effects on development of diseases.

Ellagitannins (ETs) and ellagic acid (EA) are natural polyphenols found in fruits and nuts, as well as some traditional Chinese medicines. The physical and chemical properties of ETs with high molecular weight and strong polarity determine their low bioavailability. The micro-ecological balance of intestinal microbiota affects energy absorption from food and immune system, thus affecting human health. As metabolites of intestinal flora, urolithins have many biological activities, such as anti-oxidation (*8*), anti-inflammation (*9*), inducing fat browning (*10*), and regulating lipid metabolism (*11*). In recent years, the regulatory effects of urolithins on intestinal microbiota and intestinal inflammatory diseases have also received widely attention (*12*). Here, we review the origin of urolithins, urolithin-producing microorganisms and the regulatory effects on intestinal related diseases.

2. The origin of urolithins

Urolithins (uros) are polyhydroxyl derivatives of diphenylpyran-6-one, which can be considered as a combination of coumarin and isocoumarin in chemical structure. Although uro-M5 has been reported to be isolated from plants *Terminalia* (*13*), *Rosa chinensis* (*14*), *Lagerstroemia speciosa* (*15*), *Punica granatum* (*16*), *Mallotus furetianus* (*17*), and uro-A from pomegranate (*18*), uros are not common in nature.

After eating food rich in ETs, most of them are first metabolized to EA in stomach and small intestine of mammals. Then, EA loses a lactone ring to obtain uro-M5 under the action of esterase and decarboxylase in intestinal microbiota, and then gradually loses hydroxyl groups under the action of dehydroxylase to form a class of internal metabolites with different hydroxyl substitutions (*19,20*). Uro-A and uro-B were first isolated from sheep kidney stones as EA metabolites (*21*), and then were found in urine, feces, bile, prostate, colon and milk of human, rat, mouse, cow, pig, beaver and other animals. Compared with ETs and EA, urolithins are more easily absorbed in colon and can be detected in blood a few hours later under the action of intestinal microbiota. After that, it is widely distributed in the cells of the body or enters the liver with the blood circulation to participate in phase II metabolism, which is gluconic acidified, sulfated or methylated to further exert biological effects (*22,23*). The concentrations of phase II metabolites in human plasma were uro-A glucuronide with 0.024-35 μM, iso-uro-A glucuronide with 0.0045-0.745 μM and uro-B glucuronide with 0.012-7.3 μM (*24*), respectively. With the application of high-throughput and high-sensitivity detection methods, more and more urolithins and their derivatives have been discovered and studied. Members of the urolithin family include uro-M5, uro-D, uro-M6, uro-E, uro-C, uro-M7, iso-uro-A, uro-B, uro-A, uro-M6R, uro-M7R, uro-CR and uro-AR (Figure 1) and their corresponding phase II metabolites (*25,26*). According to final metabolic products, it is divided into three metabo-types including UM-A (producing only uro-A conjugates), UM-B (producing uro-A, isouro-A and/or uro-B) and UM-0 (no urolithins) (*27,28*). Uro-AR exists in both UM-A and UM-B metabo-types (*25*). Studies have shown that the urolithin producing ability and the metabo-types are not closely related to food sources, age and health status (*27*), but are determined by the intestinal microorganisms that can metabolize ETs and EA. However, the analysis of the metabo-types of 839 healthy people aged from 5 to 90 showed that 70-80% of healthy young people aged 5-30 were UM-A, 10-20% were UM-B, while UM-B type increased in people aged 30-90 (*29*). In addition, individual health status such as obesity, colon cancer, hyperlipidemia, cardiovascular disease also affects metabo-types. Romo-Vaquero *et al.* analyzed the intestinal microflora of 249 healthy individuals by 16S rDNA sequencing. The results showed that bacteria *Coriobacteriaceae* may be the relationship between the level of UMs and blood cholesterol. From the current research, UM-A may be more conducive to health, while UM-B may be associated with some diseases and flora disorders (*30*).

3. Urolithin-producing strains from intestinal microorganisms

Urolithins have been found in many animals such as mice, rats, beavers, sheep, cattle and humans after eating foods rich in ETs (*31,32*). Recently, urolithin-producing microorganisms have also been reported. The transformation of ETs and EA by fecal microorganisms of 6 volunteers in anaerobic environment was studied. Uro-A was detected in the fermentation products by fecal bacteria of different volunteers, which confirmed that uro-A is the metabolite of ETs *in vitro* for the first time, but the concentrations and yields were different, indicating that the composition of individual fecal flora was different (*33*). Studies have also been carried out on the isolation of microorganisms which can convert EA into urolithins from the feces of healthy people. Selma *et al.* confirmed for the first time that the new species *Gordonibacter urolithinfaciens* DSM27213 and *G. pamelaeae* DSM19378 have the ability to convert EA to urolithins in stationary culture under anaerobic conditions *in vitro*, and HPLC-DAD-MS analysis showed that pentahydroxy uro-M5, tetrahydroxy uro-M6 and trihydroxy uro-C were produced sequentially, but uro-A and uro-B were not detected in pure culture. It is suggested that the UM-A or UM-B

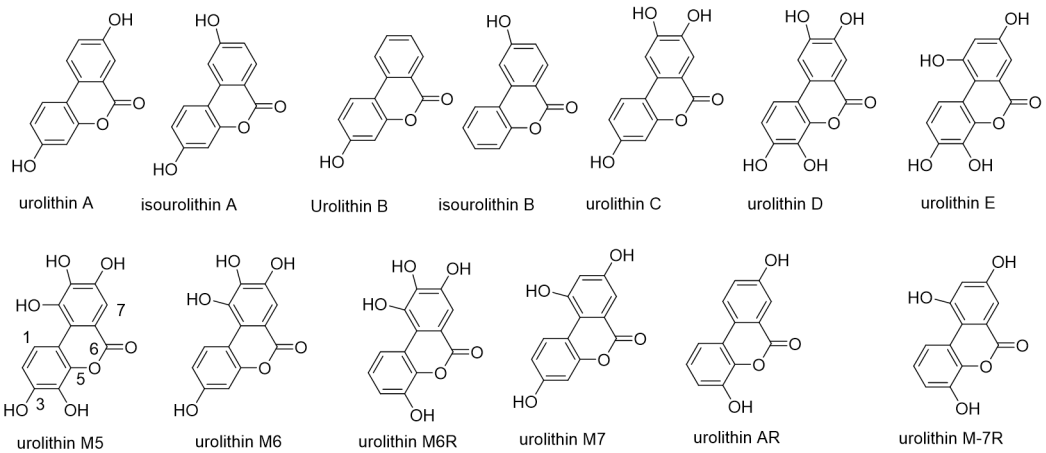

Figure 1. The presentative structures of urolithins.

metabolism may require the participation of other microorganisms or the regulation of culture conditions (*34-36*). Strains *Ellagibacteris isourolithinifaciens* DSM104140 and *G. urolithinfaciens* DSM27213 could convert EA into uro-M5, uro-M6, uro-C and isouro-A (*37*); 48 strains of *Bifidobacteria* and 1070 strains of other bacteria were isolated from the feces of healthy women and their ability to produce urolithins were tested, only strain *Bifidobacterium pseudocatenulatum* INIA P815 could convert EA into uro-A in Brain-Heart Infusion (BHI) medium (*38*).

The number of bacteria in human intestinal tract is about 10 times that of human cells, obviously, the study of microorganisms (genes or enzymes) involved in the transformation of EA *in vivo* is not deep enough. The key rate-limiting steps of urolithin production *in vivo* have not been solved (*20*). The future research should combine the traditional microbial isolation and microbial culture technology together with metagenomics and culturomics to determine the key genes or enzymes involved in the production of urolithins and carry out *in vivo* investigation, and further to explore the role of these microorganisms and enzymes in the regulation of intestinal flora and their effects on human health (*39*).

4. Urolithins and intestinal health

ETs and EA are polyphenols present in a variety of fruits, vegetables, nuts and medicinal plants, with a variety of biological activities. Most of EA is metabolized by intestinal flora to produce a series of urolithins that are more easily absorbed, and their concentrations in different tissues ranging from 0.003 to 50 μM (*40*). Therefore, urolithins may be the real bioactive substances rich in ETs in organisms. Uro-A has been reported as a potential molecule in regulating metabolic diseases such as neuro-inflammation, cardiovascular disease and obesity (*41*). Anti-oxidation, anti-inflammation, anti-cancer, anti-obesity and neuroprotective activities have been reported (*42*). Also, uro-A showed protective potential in the gastrointestinal inflammatory diseases such as CRC and inflammatory bowel disease (IBD) (*12*). Here, we mainly summarize the role of urolithins in regulating intestinal flora and intestinal health.

4.1. Urolithins inhibit bacterial infection

The virulence factor of bacteria is the guarantee for the stable existence of intestinal flora in human digestive tract and against human immunity (*43*). Inhibiting virulence is an important way to control pathogen infection. Four μM uro-A and uro-B can reduce the levels of N-hexanoyl-L-homoserinelactone (C6-HSL) and N-(3-oxohexanoyl)-L-homoserinelactone (3-oxo-C6-HSL) in *Yersinia enterocolitica*, thus inhibit the formation of quorum sensing-related biofilm and the movement ability of bacteria, and maintain the balance of intestinal flora (*44*). Uro-M5 is an inhibitor of type three secretion system of Salmonella and can protect the host by reducing virulence and inflammation (*45*). Moreover, uro-A, uro-B and uro-D have certain antibacterial activity, but their antibacterial activities are weak, coupled with their weak cytotoxicity, the researchers attributed their antibacterial activity to high intake (*46*). The above results suggested that urolithins are inhibitors of bacterial virulence without killing pathogens, and are substitute antibiotics without producing drug resistance (*47*).

4.2. Urolithins regulate intestinal flora

The intestines of human contain 100 trillion viable bacteria, including beneficial and harmful to human health. After feeding uro-A to colitis mice for 10 days, the abundance of the beneficial bacteria *Lactobacillus*, *Bifidobacterium* and *Clostridium* in fecal samples significantly increased (*48*). Also, the abundance of *Akkermansia* and *Gordonibacter* in intestinal flora of uro-A producers was higher than that of non-uro-A producers (*49*). The body weight of high-fat diet induced obese mice was greatly reduced by treatment with 2.5 mg/kg uro-A or uro-B. 16S rDNA sequencing analysis showed that the anti-obesity effects of uro-A or uro-B may play an important role in weight loss by regulating intestinal flora (*50*). Uro-A can also help restore colon tissue damage and regulate intestinal flora, thereby reducing inflammation (*51*). Food-derived metabolites can also regulate intestinal flora (*24*). Medicinal edible plants rich in ETs have different metabo-types (UM-A, UM-B and UM-0) after intestinal flora metabolism, and different metabo-types also reflect the differences of intestinal flora. Foods rich in ETs can increase the abundance of urolithin-producing *Gordonibacter* in fecal microorganisms (*52*). High-fat diet caused intestinal flora disorder in mice, in which *Ruminococcus* increased significantly. Compared with high-fat diet, foods rich in polyphenols increased the abundance of *Roseburia* and decreased the abundance of *Mogibacteriaceae,* while the polyphenols in red raspberry seeds increased the abundance of *Bifidobacterium* (*53*). Therefore, ET-containing food and urolithins can increase the beneficial bacteria such as *Akkermansia* and *Bifidobacteria*, and can restore normal intestinal balance and produce beneficial effects to maintain intestinal homeostasis.

4.3. Urolithins enhance the function of intestinal barrier

Uro-A showed anti-inflammatory activity against mouse Raw264.7 macrophages induced by lipopolysaccharide (LPS). Uro-A pretreatment and post-treatment of DSS induced colitis mice can reduce

inflammatory signals and up-regulate the expression of tumor suppressor genes, thus alleviating colonic injury and playing an important role in regulating the balance of intestinal flora in mice (*48*). Aromatic hydrocarbon receptor (AhR) plays an important regulatory role in enteritis. Recent studies on inflammatory cell models have shown that uro-A can improve the biosynthesis of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) by activating the AhR-Nrf2 dependent pathway, up-regulate the expression of tight junction proteins, prevent inflammation, and improve the barrier function of intestinal epithelium in IBD disease (*54*). Uro-A and its synthetic analog UAS03 enhance the barrier function of intestinal epithelial cells by up-regulating Nrf2 dependent epithelial cell connexin, and protect IBD by reducing inflammatory response (*55*).

4.4. Urolithins inhibit CRC and IBD

Urolithins are mainly produced in the small intestine and colon. Therefore, urolithins are expected to play a role in the intestine and intestinal wall (*56*). *In vitro* studies have proved that uro-A has the inhibitory effects on CRC and IBD. The anticancer effect of uro-A may come from autophagy induction, because autophagy will be triggered after eating polyphenols, thus inhibiting the growth and metastasis of CRC cells (*57*). The mixture of uro-A and uro-B acts on colorectal adenocarcinoma Caco-2 cells both in the long and short term, and the drug reaching the intestinal cavity helped to reduce oxidative stress, and prevented the damage caused by reactive oxygen species (*58*). Uro-A has a significant inhibitory effect on the growth of colon cancer cell line HCT116 with IC_{50} of 19 μM (72 h) and has a synergistic effect with oxaliplatin, which can induce the stability of p53 and the expression of p53 target gene, resulting in p53/p21 dependent aging like growth arrest (*59*). At an achievable concentration in the human colon and rectum, uro-A can enhance the sensitivity of 5-fluorouracil to the anticancer effect of human colon cancer cells, block the cells at G2/M and cause the activation of caspases 8 and 9 (*60*).

IBD is a chronic disease that causing inflammation in the small or large intestines, and is thought to increase the risk of CRC. Uro-A has been reported to prevent the intestinal inflammation by attenuating the inflammatory signaling and upregulating of the tumor suppressor genes (*61*), to increase the permeability of tight junctions (*62*), and to prevent the detrimental effect of inflammation on the cells' viability (*63*). Those finding have given evidence of urolithins, especially uro-A, in the protection of intestinal diseases such as CRC and IBD.

5. The safety of urolithins

Urolithins are the metabolites of tannic polyphenols *in vivo*, which exist in blood, urine and feces in a free form or phase II conjugation, and have extensive biological activities *in vivo* and *in vitro*. Therefore, the experiments based on direct oral administration can verify their safety. The genetic and toxicological toxicity of oral uro-A in rats were studied. The results suggested that high-dose oral synthetic uro-A did not show any toxicity to the target organs at the histopathological level, indicating the clinical safety of uro-A (*64*). Andreux *et al.* recruited 60 elderly people and randomly divided them into four groups: placebo group, uro-A 250 mg, 500 mg and 1,000 mg daily for 28 days. The effects of uro-A on the body were evaluated by the levels of health biomarkers of cells and mitochondria in blood and muscle tissue. The results indicated that uro-A can help slow down the aging process by improving the function of cell mitochondria. It was also found that intake of uro-A had no risk to human health (*65*). And now, the safety evaluation of urolithin *in vivo* is limited to uro-A. So, many problems need to be studied, such as the safety and biological activity of other uros to human body, whether they can enter the blood-brain barrier, their existing form and concentration, the specific function in human body and the relationship with human health.

6. Conclusions

Intestinal microbiota regulates the material and energy metabolism of the host, "You are what you eat" (*66*). Different eating habits have a great impact on the types of intestinal microorganisms in human. At the same time, the types of intestinal microorganisms also determine the metabo-types. At present, the production process of urolithins *in vivo*, the mechanism of intestinal diseases and the interaction with intestinal flora are still in the exploratory stage. It is of great significance to analyze the pharmacological effect and mechanism of urolithins *in vivo* through metabonomics, culturomics and microbiomics, to explore the development of relevant microbial preparations and drugs, and promote its application in the prevention and treatment of intestinal diseases.

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