Facing a new challenge: the adverse effects of antibiotics on gut microbiota and host immunity

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Mammals have existed on Earth for millions of years. During this long period, mammals and their gut commensal microbes have coevolved and coadapted, forming a complex and inseparable interrelationship called "superorganisms." On the one hand, the food and gut environment play an important role in shaping the composition and function of gut microbiota. On the other hand, gut microbiota actively participates in host nutrition metabolism and profoundly affect host immunity. However, this natural coevolving process has been significantly changed in recent decades by antibiotic administration. Although antibiotic therapy is considered as a milestone in fighting infectious diseases, its negative effects on gut microbiota and host health have been recognized. Here, we will discuss the unfavorable effects and underlying mechanism of antibiotics on gut microbiota and host immunity, as well as possible solutions to reverse adverse effects brought about by antibiotic treatment.

An overview of gut microbiota

The gut is the largest reservoir for microbiota in mammals and humans. However, our understanding of gut microbes has been limited for a long time because most gut microbes cannot be identified by traditional culture technology. Recently, the rapid development of high-throughput sequencing technology, including metagenomics, metatranscriptomics, and metaproteomics approaches, has provided us with powerful tools to study the composition and function of gut microbiota. It is estimated that approximately 100 trillion microbes from over 1000 species and more than 7000 strains reside in the gut. Although enormous gut microbes in addition to bacteria have recently been identified (including helminths, protozoa, archaea, viruses, phages, yeast, and fungi), bacteria are still the main participants in gut and host homeostasis. The five major bacterial phyla in the gut are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, accounting for more than 90% of the total bacterial population that inhabit the gut. The rest of bacteria are from less abundant phyla, such as *Fusobacteria* and *Fibrobacteres*.[1] These microbial communities reside with varied density in different segments of the gut and play a crucial role in many aspects of physiological processes, including facilitating food digestion and energy utilization, synthesizing vitamins and essential amino acids, promoting the development of the immune system, maintaining the integrity of the gut mucosal barrier, and protecting against enterogenous pathogens.[1]

Antibiotic administration leads to gut microbial dysbiosis-associated diseases

Since Alexander Fleming discovered the first antibiotic (penicillin) in 1928, thousands of antibiotic substances have been extracted from natural substances or have been artificially synthesized. The emergence of antibiotics has protected humankind from assaults from various pathogenic bacteria and saved millions of lives during the last century. However, extensive use of antibiotics also negatively impacts human health. A growing number of studies have shown that antibiotics can result in microbial dysbiosis, and the disruption of gut microbiota in neonates and adults contributes to numerous diseases, including diabetes, obesity, inflammatory bowel disease, asthma, rheumatoid arthritis, depression, autism, and superinfection in critically ill patients.

The direct and indirect effects of antibiotics on gut microbiota

Antibiotics can affect gut microbiota through direct or indirect mechanisms. Antibiotics are intentionally

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administered for the depletion of pathogenic bacteria; however, due to their broad-spectrum activities, subsets of commensal microbes are also indiscriminately killed or inhibited. Notably, different antibiotics or their combinations have different antimicrobial spectra and will result in different changes to the microbiome. For example, vancomycin decreases fecal microbial diversity and the absolute number of gram-positive bacteria, particularly the *Firmicutes* phylum, whereas amoxicillin does not change total bacterial numbers and microbial diversity significantly. A combination of antibiotics containing ampicillin, gentamicin, metronidazole, neomycin, and vancomycin not only reduced the total number of bacteria but also dramatically shifted the composition of gut microbiota.[2,3] Therefore, when we use antibiotics as a tool to study the effects of microbiota on host health, we should first choose appropriate antibiotics and understand how the chosen antibiotics will reshape the gut microbiome.

Beyond the direct effect, antibiotics can also indirectly impair gut microbiota. Symbiosis and codependency are universal among different subsets of gut microbiota. Under normal physiological conditions, the microbiota maintains a homeostatic state. The secondary metabolites produced by some species of microbiota may be necessary nutrients for other colonizers. For example, *Bifidobacterium adolescentis* are able to utilize fructooligosaccharides and starch to produce lactate and acetate. Butyrate-producing anaerobes cannot directly utilize fructooligosaccharides and starch but can utilize lactate and acetate as growth substrates. Therefore, *B. adolescentis* can facilitate the proliferation and expansion of butyrate-producing species *in vivo* by cross-feeding. This cross-feeding-dependent symbiotic relationship is also found in *Rhodopseudomonas palustris* and *Escherichia coli*, *Methanobrevibacter smithii* and *Bacteroides thetaiotaomicron*, and *Eubacterium rectale* and *B. thetaiotaomicron*.[4] Otherwise, some metabolites accumulated in the gut may be toxic to other microbes, and microbial biotransformation of these toxic metabolites may be restricted to specific species. One of the most striking examples is conjugated bile acids, which can inhibit the growth of bacteria in the duodenum and jejunum. Deconjugation by *Lactobacilli*, *Bifidobacteria*, *Clostridium*, and *Bacteroides* is the key step in reducing the toxicity of bile acid. Deconjugated bile acids can be further used by bacteria or reabsorbed by the liver for bile acid enterohepatic circulation.[5] Thus, loss of specific populations of microbiota may lead to the alteration of metabolites and the microenvironment in the gut, which in turn affect the growth of other members of the gut microbiota.

The negative effects of antibiotics on gut microbiota and host immunity

Increase in antibiotic-resistance genes

Antibiotics can cause a number of unfavorable effects on host health. One direct adverse effect is that the administration of antibiotics can drive the generation and spread of antibiotic-resistance genes in gut microbiota. Under selective pressure by antibiotics, sensitive strains will be eliminated, giving the antibiotic-resistant strains a growth advantage. Then, antibiotic-resistance genes can be horizontally spread among bacteria through three kinds of mechanisms: conjugation, transduction, and transformation.[2] Among all the horizontally transmitted genes between diverse bacterial species in the human gut microbiome, the percentage of transmitted antibiotic-resistance genes is approximately 6%, which is 4.8-fold higher than the percentage of transmitted antimicrobial peptide-resistance genes.[6] Moreover, antibiotic-resistance genes are more readily transmitted across bacterial species, which may lead to a rapid dissemination of antibiotic resistance in other members of the gut microbiota.

Change in bacterial metabolites

Antibiotics also impact host immunity by altering the bacterial metabolites and the signals transmitted from gut microbiota to the host, especially the signals recognized by intestinal epithelial cells and intestinal immune cells. Metabonomic analysis has revealed that antibiotics can have a profound effect on lipids, bile acids, amino acids, and amino acid-related substances in the gut. Short-chain fatty acids (SCFAs), produced by bacteria in the large intestine through fermentation of fibers, have broad effects on enterocytes, including maintenance of epithelial integrity, regulation of Treg differentiation and accumulation, and modulation of inflammatory and immune responses.[2,3] Depletion of commensal bacteria by antibiotics leads to a reduced production of SCFAs, a lower frequency of Th17 and Treg cells, and increased gut inflammation during oral *Candida albicans* infections in mice. SCFA administration promotes *C. albicans* clearance and inflammation resolution in antibiotic-treated mice.[7]

Clostridium difficile, a spore-forming gram-positive anaerobic bacteria and the leading cause of antibiotic-associated diarrhea, is significantly inhibited by secondary bile acids. A combination of cefoperazone, clindamycin, and vancomycin is associated with the loss of *Lachnospiraceae* and *Ruminococcaceae* families and a reduced transformation of primary bile acids to secondary bile acids in the large intestine, increasing the risk of *C. difficile* infection. Otherwise, antibiotic-induced alteration of amino acids, especially proline, has been identified as an important factor for *C. difficile* colonization.[8]

Disrupted bacterial signaling and antimicrobial peptide secretion

Despite the impact of antibiotics on bacterial metabolites, the impact of antibiotics on the interaction between the host and the gut microbiota is more profound and comprehensive. Gut microbes constantly communicate with the host through the activation of host pattern-recognition receptors (PRRs), including toll-like receptors (TLRs) and NOD-like receptors. Depletion of gram-negative bacteria with antibiotics reduces TLR4- and MyD88-mediated signaling, resulting in a diminished expression of Reg3g, a secreted C-type lectin antimicrobial peptide that kills gram-positive bacteria. Clearance of vancomycin-resistant *Enterococci* (VRE) is compromised in mice with gram-negative commensal bacteria depletion. Oral administration of LPS (the agonist of TLR4) restores

the production of Reg3g and corrects the VRE-clearance defect in the intestinal lumen.[2] Depletion of gram-positive bacteria decreases TLR2 activation, which diminishes the expression of Reg3β, another C-type lectin antimicrobial peptide targets specific gram-negative bacteria. Reg3β-deficient mice have impaired defense against gram-negative *Salmonella* translocation and dissemination in host tissues.[3] NOD1, which recognizes peptidoglycan mainly derived from gram-negative bacteria, plays an important role in priming neutrophil function and enhancing its killing of *Streptococcus pneumoniae* and *Staphylococcus aureus*. Depletion of the gut microbiota by antibiotics decreases the concentration of peptidoglycan derived from gram-negative bacteria and correlates with impaired ability to kill *S. pneumoniae* and *S. aureus*. Administration of NOD1 ligands restores neutrophil function and protects mice against early pneumococcal-induced sepsis.[2] NOD2 can recognize peptidoglycan derived from gram-negative and gram-positive bacteria and induce the expression of α-defensins (an antimicrobial peptide) in Paneth cells. The presence of gut commensal microbiota is required for NOD2 expression, and NOD2 deficiency is associated with increased susceptibility to *S. pneumoniae*, *S. aureus*, and *Listeria monocytogenes* infections in mice.[3]

Gut immune cell dysregulation

Antibiotic-induced perturbation of the microbiota not only affects mucosal PRRs signaling and the secretion of antimicrobial peptides in the gut but also compromises the development and function of gut immune cells. Tissue-resident group 3 innate lymphoid cells, which are prevalent in the intestinal lamina propria, have been demonstrated to be essential for retaining microbes in the gut lumen and preventing bacteria translocation via an interleukin (IL)-22-dependent pathway. Depletion of gut microbiota with antibiotics has a profound impact on group 3 innate lymphoid cells recruitment and development, resulting in reduced IL-22 production and rendering the host more susceptible to invading pathogens.[3] Otherwise, disruption of the gut microbiota by antibiotics can also affect other groups of gut immune cells, manifesting as a dysregulated ratio of type 1 T-helper cells to type 2 T-helper cells, a perturbed differentiation of naive T cells into regulatory T cells, and a reduced frequency of type 17 T-helper cells. All these changes alter gut immune homeostasis and may confer susceptibility to enterogenic infection.[3]

Host systemic immunity dysfunction

Notably, antibiotic-induced perturbation of immune homeostasis is not restricted to the gut. Recent studies have demonstrated that the depletion of resident microbiota also affects systemic immunity. Administration of antibiotics to maternal mice decreases IL-17A production, plasma granulocyte-colony stimulating factor levels, and the numbers of neutrophils in bone marrow and circulation in neonatal mice, which is associated with increased susceptibility to *E. coli* K1 and *Klebsiella pneumoniae* sepsis in the early neonatal period. Depletion of microbiota with antibiotics also impairs the pulmonary defense against pathogens. Microbiota depletion leads to altered metabolism within alveolar macrophages, which correlates with a diminished capacity to phagocytose *S. pneumoniae* and a compromised response to lipopolysaccharide and lipoteichoic acid stimulation. Moreover, a loss of gut microbiota mediates the dysregulation of TLRs signaling, which also causes a reduced expression of a proliferation inducing ligand (APRIL) and decreased pulmonary immunoglobulin A (IgA) production in mice and critically ill patients. As IgA can bind to *P. aeruginosa* and enhance its clearance, a secondary IgA deficiency in the lungs caused by antibiotic treatment predisposes the host to *P. aeruginosa* pneumonia.[9] Otherwise, gut-derived IgA+ plasma cells can access the central nervous system and attenuate neuroinflammation via a IL-10-dependent manner in mouse models and in patients with multiple sclerosis.[10] Depletion of gut microbiota by antibiotics also affects the host's adaptive immunity against hepatitis B virus (HBV) infection. Patients treated with antibiotics showed a decreased interferon-γ (IFN-γ) production and an impaired HBV clearance.[11]

Strategies to reduce antibiotic-induced gut microbial dysbiosis and immune disorders

Because antibiotic administration elicits many adverse effects, as mentioned in this review, measures taken to restrict the overuse of antibiotics are imperative. In a recent meta-analysis that included 6708 patients from 26 eligible trials in 12 countries suggested that the implementation of procalcitonin protocols to guide antibiotic treatment in patients with acute respiratory infections has the potential to reduce antibiotic exposure and side effects and improve survival.[12] Despite procalcitonin, other biomarkers or clinical algorithms, such as the C-reactive protein-based algorithm, clinical pulmonary infection score evaluation, routine bronchoscopy, and microbiologic examinations, have also been demonstrated to be effective in reducing antibiotic exposure. However, it is unrealistic to completely abandon antibiotics in clinical practice, especially for patients with severe infections. Therefore, several strategies have been proposed to attenuate antibiotic-induced microbial dysbiosis and immune disorders, although most of the studies have been carried out under laboratory conditions. The first strategy is to administer PRRs agonists locally or systemically. Oral administration of LPS (the agonist of TLR4) partially reverses postnatal granulocytopenia through a TLR4- and MyD88-dependent pathway in neonatal mice receiving antibiotics.[2] Systemic administration of flagellin (the agonist of TLR5) or oral delivery of resiquimod (a synthetic ligand of TLR7) can upregulate the expression of Reg3g via a IL-22-dependent pathway, thereby enhancing the defense against VRE infection in antibiotic-treated mice.[3] However, administration of a certain PRRs agonist cannot restore the complex signaling network orchestrated by various resident microbes in the gut. Thus, the second method is to use bacterial lysates or products that contain multiple PRRs ligands to restore immune homeostasis. It has been demonstrated that the incubation of peripheral B cells (CD19+) and T lymphocytes (CD3+) with gut-resident antigen-presenting cells (APC)^{TNLG8A} and gut microbial products at a physiological aerobe-to-anaerobe ratio of 1:100 can markedly induce the expression of signaling

lymphocyte-activation molecule family member 4, which is a marker of intestinal immune cells and contributes to protecting the host against enteric pathogens, such as *L. monocytogenes* and *Citrobacter rodentium*.[13] The third strategy is the oral administration of live probiotics to restore the gut microbiota balance. The genera *Lactobacillus*, *Saccharomyces*, *Bacillus*, *Bifidobacterium*, and *Enterococcus* are the five most commonly used probiotics. Although experimental studies suggest that hosts may benefit from probiotic intake through several mechanisms, including the inhibition of the growth of pathogens by competition for space and nutrients, the stimulation of host immunity, the induction of antimicrobial peptides, and the maintenance of the integrity of the epithelial barrier, the effectiveness of probiotics in preventing or treating antibiotic-induced microbial dysbiosis is still controversial. A comprehensive meta-analysis that included 82 randomized controlled trials suggested that probiotic intervention is associated with a reduced risk of antibiotic-associated diarrhea; however, due to the significant heterogeneity among different subgroups, this conclusion should be interpreted with caution. A recent study suggested that probiotics may specifically prevent *C. difficile* infection in patients receiving antibiotics.[14] The fourth method is gut microbiota transplantation. Fecal microbiota transplantation (FMT) can control intestinal inflammation and restore intestinal homeostasis through multiple mechanisms, including increasing IL-10 production by CD4+ T cells, iNKT cells, and APCs, restoring secondary bile acid metabolism, providing signals for epithelial regeneration, and stimulating antimicrobial peptide secretion. FMT has been proven to be successful in the treatment of recurrent or refractory *Clostridium difficile* infection, with a reported cure rate of 87% to 90%. More specifically, selective transfer of a single species of bacteria, *Clostridium scindens*, is sufficient to increase host resistance to *C. difficile* infection because *C. scindens* can convert primary bile salts to secondary bile salts, which serve as potent inhibitors for *C. difficile* colonization.[15] It is still unknown whether microbiota transplantation can be as effective in the treatment of other superinfections as it is with *C. difficile*, but microbiota transplantation is a promising candidate that may help us to counteract the negative effects of antibiotics on gut microbiota and host hemostasis.

Future expectation

With the rapid development of microbial omics technologies, our understanding of the negative effects of antibiotics on host intestinal microbiota and immune system will become more comprehensive. In consideration the broad impact of antibiotics on gut microbiota, future studies need to evaluate the effect of antibiotics on composition and functionality of gut microbiota and host immunity. Probiotics and FMT are two promising therapeutic methods in the management of antibiotic-induced gut microbial dysbiosis. However, long-term follow-up concerning safety issues, the impacts of probiotics and FMT on intestinal microbiota and host immunity, and the impacts on nutrients metabolism remain to be evaluated in future studies.

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Conflicts of interest

None.

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