

## Review



# Complex influences of gut microbiome metabolism on various drug responses

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## ABSTRACT

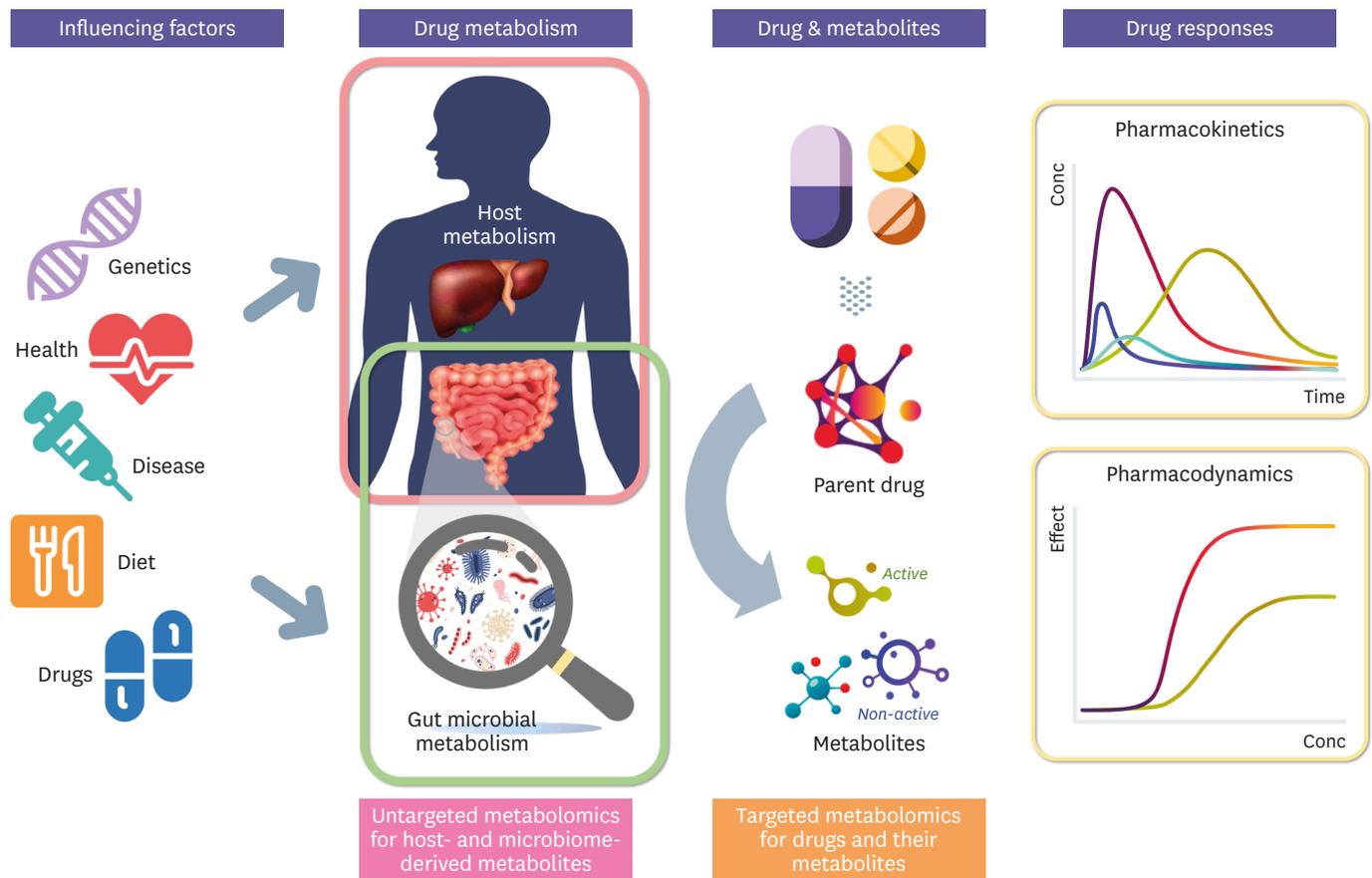
The gut microbiome closely interacts with the host, and it has a major influence on drug response. Many studies have reported the possible microbial influences on drugs and the possible influences of drugs on the microbiome. This knowledge has led to a better understanding of intra- and inter-individual variabilities in clinical pharmacology. For a more precise understanding of the complex correlation between the microbiome and drugs, in this review, we summarized the current knowledge on the interactions between the gut microbiome and drug response. Moreover, we suggest gut microbiome-derived metabolites as possible modulators of drug response and recommend metabolomics as a powerful tool to achieve such understanding.

**Keywords:** Microbiome; Drug effects; Metabolism; Metabolomics

## INTRODUCTION

Over the last several decades, various factors have been considered to play a role in drug response, including drug efficacy and adverse effects. In addition to the influences of pharmacogenomic variations, various environmental and pathophysiological factors, microbial factors, and co-administration of xenobiotics have been reported to affect the intra- and inter-variability in the pharmacokinetics and pharmacodynamics of many drugs (**Figure 1**). Recently, many researchers have reported the association of diseases and drug responses with the gut microbiota composition and function. For example, the Human Microbiome Project carried out by the National Institutes of Health, USA revealed an association between drug responses and gut microbiome composition [1]. Such studies have increased the interest of clinical pharmacologists to seek knowledge regarding how the human gut microbiome interacts with therapeutic drugs, which may enable better drug selection and dosing.

Orally delivered drugs are metabolized and absorbed in the gastrointestinal tract prior to the systemic circulation. The gastrointestinal tract hosts a number of microbial communities, which confer several benefits to the host, such as aiding digestion of food and biosynthesis of vitamins. The gastrointestinal tract also hosts enzymes and metabolites secreted by



**Figure 1.** Overview of drug metabolism throughout the host and the gut microbiome. Orally administered drugs are metabolized by hepatocytes, enterocytes, and other cells of the body as well as by the gut microbiome. Host- and gut microbiome-mediated drug metabolism is influenced by various factors, such as host genetics, health conditions, disease, diet, and drug use, including antibiotics and non-antibiotics. In addition to human drug metabolizing enzymes, the microbial species and enzymes are involved in the metabolism of some drugs, which can alter production of the active and non-active metabolites and further control drug responses such as pharmacokinetics and/or pharmacodynamics. Both targeted metabolomics for drugs and their metabolites and untargeted metabolomics for host- and microbiome-derived metabolites can help in better understanding the mechanisms underlying host and microbiome-related interindividual differences in drug metabolism.

the microbial communities. These microbial enzymes present in the gut lumen can also affect drug metabolism. The Human Microbiome Project has recorded approximately 3013 microbiome-encoded  $\beta$ -glucuronidases with various functional capacities in the gastrointestinal database [2]. Other studies have also reported the presence of microbial polysaccharide lyase, lipases, reductases, endoglycosidases, transferases, oxygenases, sulfatases, and glycol radical enzymes in the gut [3,4]. For example, digoxin is inactivated by the cardiac glycoside reductase (*cgr*) of *Eggerthella lenta* [5], and levodopa is activated into dopamine by the tyrosine decarboxylase of *Enterococcus faecalis* [6]. These studies indicate that not only drugs but also their metabolites can be further metabolized by microbial enzymes. Busulfan is first metabolized into glutathione S-conjugate by direct interaction with glutathione and host glutathione S-transferase [7-10], and the metabolite that contains the cysteine-S-bond is cleaved by microbial C-S- $\beta$ -lyases and further metabolized [11]. These microbial enzymes can also directly metabolize drugs in the gastrointestinal tract to their active or inactive form. Thus, the gut microbiome affects drug metabolism or efficacy indirectly via microbial regulation of host metabolism and transportation of drugs and their metabolites or by altering host immune responses [12].

Interactions between drugs and microbial activities hold the potential of being an important clue for further understanding of differences in drug responses. In this review, we briefly summarized the current knowledge of the interactions between the gut microbiome and drug response. Moreover, we suggest gut microbiome-derived metabolite(s) as the possible modulator(s) of drug response and recommend metabolomics as a powerful tool to achieve such understanding.

## MICROBIAL INFLUENCES ON DRUG RESPONSES

Drug responses are influenced by the host microbiome. For example, acarbose, an  $\alpha$ -glucosidase inhibitor, is negatively correlated with increased *Lactobacillus* and *Dialister* spp. and blood glucose levels [13]. Drugs other than antidiabetic agents also have been shown to be associated with the microbiome. Digoxin, which is used to treat heart failure, is affected by the *cgr* gene cluster identified in specific strains of *E. lenta*. The *cgr* operon that encodes two proteins enables certain strains of *E. lenta* to metabolize digoxin into dihydrodigoxin, leading to decreased exposure of the host to digoxin [14,15]. *Bacteroides* also produce (E)-5-(2-bromovinyl) uracil metabolite, which increases the toxicity of sorivudine [16]. Statins are known to have a correlation with both bile acids and microbiome. For example, the increased plasma concentration of simvastatin is positively correlated with higher levels of several secondary bile acids produced from the gut microbiome [17]. Also, rosuvastatin reduces the hepatic expression of CYP27a1, resulting in a decreased level of cholic acid and chenodeoxycholic acid [18]. Microbial composition and rosuvastatin have been reported to have a bilateral correlation. In detail, higher complexity in the microbial composition is positively correlated with rosuvastatin's efficacy on low-density lipoprotein-cholesterol [19], and rosuvastatin treatment has been reported to lower the species richness and phylogenetic diversity [18]. Responses of immune checkpoint inhibitors to the gut microbiome have also been studied. *Bacteroides caccae* has been shown to have a positive effect on several types of immuno-cancer therapeutic agents [20].

## MICROBIAL CHANGES INDUCED BY ANTIBIOTICS

Orally administrated antibiotics are often used as concomitant medication to treat or prevent infection, especially in the digestive system. A common concern while administering concomitant medication is drug–drug interaction. However, it is also important to consider the effects of concomitant medication on the gut microbiome, which may affect the metabolism of the primary drug. Most antibiotic agents have a wide target concentration range, eventually disrupting gut microbiota and affecting non-pathogenic organisms, leading to the risk of long-term or permanent loss of certain members of the gut microbiota. In this review, we elaborate on the three most commonly used antibiotic agents and summarize the findings of the agents.

Vancomycin is used to treat various infections caused by gram-positive bacteria and has been widely studied. Some reports have confirmed that vancomycin can alter gut microbiome composition [21-23]. Oral treatment of vancomycin has been reported to decrease the species richness and diversity indices [24]. Sun et al. [24] reported that the relative abundance of Bacteroidetes, Firmicutes and Melainabacteria decreased in mice fecal samples after 3 weeks treatment of vancomycin when compared with non-treated mice (5.5% vs. 59.3%, 10.4%

vs. 37.1%, and 0.06% vs. 0.2%, respectively). As the gut microbiota itself can be described as a community of co-dependence, disruption of the existing balance by the anti-biotic effect of vancomycin on gram-positive commensal bacteria can cause long-term side effects. Vancomycin treatment indirectly affects species other than its own specific target, which exchange secondary metabolites or waste products [25,26]. Another study reported that oral vancomycin treatment led to a decrease in the abundance of Bacteroidetes and an increase in the abundance of Proteobacteria. This study also reported changes in the fecal metabolic profile, such as increased levels of D-glucuronic acid and L-phenylalanine and decreased levels of nicotinic acid and D-arabinose. The change in the levels of D-glucuronic acid and L-phenylalanine was found to be positively correlated with the abundance of Firmicutes and Proteobacteria, and negatively correlated with the abundance of Bacteroidetes. In contrast, decreased nicotinic acid and D-arabinose levels were positively correlated with the abundance of Bacteroidetes, but negatively correlated with the abundance of Firmicutes and Proteobacteria. This study suggests that treatment with antibiotics can alter the composition of the microbiota, and consequently affect the metabolomic profile [27].

Streptomycin is another commonly used antibiotic for tuberculosis, which targets *Mycobacterium tuberculosis*; it is also used to treat infective endocarditis. Following oral streptomycin treatment, a significant decrease in the total number of bacteria [28] and a marked reduction in bacterial diversity have been reported [21]. A report by Sekirov et al. [21] indicated that treatment with increasing doses of streptomycin resulted in a gradually increased proportion of Cytophaga-Flavobacterium-Bacteroidetes in a dose-dependent manner. These findings were confirmed by Grayson, who also found an increase in the number of lactobacilli and enterococci/group D streptococci and a gradual decrease in the number of Firmicutes [29].

Penicillin, a widely used antibiotic, has been reported to increase the abundance of only Proteobacteria, but not of Bacteroidetes and Firmicutes, and to decrease the abundance of Cyanobacteria and Actinobacteria [30]. Early exposure to penicillin also leads to a total elimination of Deferribacteres, with an increased proportion of Cyanobacteria. Penicillin-induced reduction in Bacteroidetes and an increase in Firmicutes and Proteobacteria have been reported to be maintained until the cessation of penicillin administration [30]. Phenoxymethylpenicillin, also known as penicillin V, causes a shift in microbiome composition such as increased phylum Proteobacteria with the dominant species *Escherichia coli*. In the other major phylum Firmicutes, the shift to Enterococcus from Streptococcus or Anaerostipes has also been reported to be induced by penicillin V treatment [31].

## MICROBIAL CHANGES INDUCED BY NON-ANTIBIOTIC DRUGS

Maier and co-workers published a report in 2018 detailing the impact of human-targeted non-antibiotic drugs on the human gut microbiome. They suggested that 24% of the human-targeted drugs in the market inhibited the growth of at least one bacterial strain *in vitro*. The susceptibility to antibiotics and human-targeted drugs correlates across bacterial species, suggesting a common resistance mechanism. These effects are largely observed with the administration of antipsychotics, including antiseptic agents. The effects of human-targeted drugs on gut bacteria are reflected in their antibiotic-like side effects in humans and are concordant with existing human cohort studies [32].

Elbere et al. [33] reported a significant reduction in the inner diversity of gut microbiota 24 hours after metformin treatment. They also reported the association between the gastrointestinal side effects of metformin and the increased abundance of common gut opportunistic pathogens such as *Escherichia-Shigella* spp. Another research team from China also reported that metformin treatment depletes Lachnospiraceae and Rhodobacteraceae abundance, but enriches Verrucomicrobiaceae and Prevotellaceae abundance [34]. Moreover, Forslund et al. [35] also reported an increase in *Escherichia*, *Lactobacillus*, *Akkermansia muciniphila* along with a decrease in *Intestinibacter* and *Bacteroides fragilis* abundance following metformin treatment. They also suggested a correlation between microbiome alterations and metformin treatment. These findings indicate that the microbial environment is affected by metformin action and *vice versa*.

A study on ursodeoxycholic acid, a secondary bile acid produced by the microbiota, suggested that ursodeoxycholic acid treatment induces changes in the microbiome in a way that can affect phenylalanine/tryptophan metabolism in patients with nonalcoholic fatty liver disease. Chao1 diversity was decreased after ursodeoxycholic acid treatment, and the beta diversity showed the difference induced by the treatment. In particular, *Bifidobacterium*, *Lactobacillus*, and *Lactobacillaceae* showed decreased relative abundance. This study also reported statistically significant changes in the metabolome profile, including increased levels of microbe-generated metabolites, along with microbiome changes [36].

## MICROBIAL METABOLITES AS THE POTENTIAL KEY TO UNDERSTAND INDIVIDUAL VARIABILITIES

The gut microbiota interacts with the host via various pathways, and the metabolites produced are involved in many well-known host pathways. Besides secondary bile acids, which are produced by bacterial reactions in the colon, lipopolysaccharides, and peptidoglycans, which are major components of gram-negative and gram-positive bacteria, respectively, enter the host liver through the portal vein [37]. In non-alcoholic fatty liver disease, these microbial products are found to activate toll-like receptors on host immune cells, thereby promoting inflammatory cytokine production. The increase in the levels of inflammatory cytokines such as tumor necrosis factor  $\alpha$  or interleukin  $1\beta$  contributes to the development of non-alcoholic fatty liver disease [38-41]. Other metabolites produced by gut microbes include short-chain fatty acids such as butyrate, acetate, and propionate. These metabolites are mainly produced by Firmicutes, Bacteroidetes, and *E. coli* [42], and get diffused [43] or transported [44,45] to the host's system. In the host's system, they exert anti-inflammatory effects on the intestinal mucosa by inhibiting histone deacetylases and activating G-protein coupled receptors [46-50].

To achieve the proper analysis and interpretation of such microbial metabolites in the host system, the metabolomic technology can be used. The application of metabolomics ranges from quantification to global untargeted metabolic profiling. For microbial metabolites study, we suggest global metabolic profiling, which can provide multiple potential target metabolites (**Figure 1**). In a previous report on ursodeoxycholic acid, global metabolic profiles were obtained from plasma and urine. These findings provided an understanding of the mechanism underlying the liver protective effects of ursodeoxycholic acid [36]. In a recent study, global metabolic profiling of patients' plasma showed that many microbial metabolites, including aryl hydrocarbon receptor ligands such as indolpropionate,

significantly changed at the onset of acute graft versus host disease [51]. This study proves that the global profile of microbial metabolites can be achieved even without fecal samples, because microbial metabolites can circulate in the host system.

Metabolomic analysis of microbial metabolites also have a definite advantage in the field of clinical pharmacology. There are many unclear underlying mechanisms in variabilities of drug response, and for further understanding, the molecular level of researches is required. A famous example of how microbial metabolites influences one's drug response is *p*-cresol. The *p*-cresol formed by Coriobacteriaceae and Clostridia [52], interfere sulfonation of acetaminophen through the production of *p*-cresol sulfate in the liver competitively, and then individuals with the higher urinary level of *p*-cresol sulfate tend to endure acetaminophen toxicity than those with a lower level of *p*-cresol sulfate in urine [53].

## APPLICATION OF METABOLOMICS IN PHARMACO-MICROBIOMICS

“Pharmaco-microbiomics” is an area that deals with how interindividual variation in the microbiome influences drug efficacy and side effect profiles [54]. Zimmermann et al. [55] performed high-throughput genetic analyses and mass spectrometry to systematically identify microbial gene products that metabolize drugs. They found that many drugs are chemically modified by microorganisms, by measuring the ability of 76 human gut bacteria from diverse clades to metabolize 271 orally administered drugs. Mallory et al. [56] have made some early progress toward establishing a computational tool for the prediction of microbial drug metabolism by applying this approach to the MetaCyc database. Recently, two research groups have reported the systematic quantification of large drug panels and their microbial metabolites using HPLC-MS followed by *ex vivo* experiments [57,58]. In spite of the remarkable progress in the field of pharmaco-microbiomics, further efforts are necessary to predict the overall effect of microbial drug metabolism. In particular, better tools, such as improved methods for quantifying microbial abundance and activities, systematically complementary computational and experimental methods, and multi-omics datasets, are required. In this context, targeted and untargeted metabolomics will help in assessing the relevance of the identified mechanisms of variability in microbiomes leading to interpersonal differences in drug metabolism.

## CONCLUSION

The gut microbiome is known to have complex interactions with drugs. Metagenomics analysis has revealed that many microbes influence drug responses directly or indirectly (through the host system). Although the influence of microbiome-derived metabolites on the host body is known, the influence of microbial metabolites on drugs is yet to be studied. To understand the correlation between microbiome and drugs clearly, we suggest the application of metabolomics to interpret the influence of microbial metabolites on drug responses. This approach bares the potential to lead us to clinical advancements in precision medicine.

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