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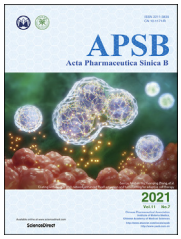

REVIEW

The influence of the gut microbiota on the bioavailability of oral drugs

Xintong Zhang, Ying Han, Wei Huang, Mingji Jin, Zhonggao Gao*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Department of Pharmaceutics, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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KEY WORDS

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Abstract Due to its safety, convenience, low cost and good compliance, oral administration attracts lots of attention. However, the efficacy of many oral drugs is limited to their unsatisfactory bioavailability in the gastrointestinal tract. One of the critical and most overlooked factors is the symbiotic gut microbiota that can modulate the bioavailability of oral drugs by participating in the biotransformation of oral drugs, influencing the drug transport process and altering some gastrointestinal properties. In this review, we

Abbreviations: 5-ASA, 5-aminosalicylic acid; AA, ascorbic acid; ABC, ATP-binding cassette; ACS, amphipathic chitosan derivative; AMI, amiodarone; an OTC drug, an over-the-counter drug; AQP4, aquaporin 4; AR, azoreductase; ASP, amisulpride; BBR, berberine; BCRP, breast cancer resistance protein; BCS, biopharmaceutics classification system; BDDCS, the biopharmaceutics drug disposition classification system; BDEPT, the bacteria-directed enzyme prodrug therapy; BSH, bile salt hydrolase; CA, cholic acid; CDCA, chenodeoxycholic acid; *cgr* operon, cardiac glycoside reductase operon; CPP, cell-penetrating peptide; CS, chitosan; DCA, deoxycholic acid; dhBBR, dihydroberberine; DRPs, digoxin reduction products; EcN, *Escherichia coli* Nissle 1917; FA, folate; FAO, Food and Agriculture Organization of the United Nations; GCDC, glycochenodeoxycholate; GL, glycyrrhizic acid; HFD, high fat diet; HTC, hematocrit; IBD, inflammatory bowel disease; LCA, lithocholic acid; LPS, lipopolysaccharide; MATEs, multidrug and toxin extrusion proteins; *MDR1*, multidrug resistance gene 1; MDR1a, multidrug resistance protein-1a; MKC, monoketocholic acid; MPA, mycophenolic acid; MRP2, multidrug resistance-associated protein 2; NaDC, sodium deoxycholate; NaGC, sodium glycholate; NEC, necrotizing enterocolitis; NMEs, new molecular entities; NRs, nitroreductases; NSAIDs, non-steroidal anti-inflammatory drugs; OATs, organic anion transporters; OCTNs, organic zwitterion/cation; OCTs, organic cation transporters; PD, Parkinson's disease; P-gp, P-glycoprotein; pK_a, dissociation constant; PPIs, proton pump inhibitors; PT, pectin; PWSDs, poorly water-soluble drugs; RA, rheumatoid arthritis; RBC, red blood cell; SCFAs, short-chain fatty acids; SGLT-1, sodium-coupled glucose transporter 1; SLC, solute carrier; SLN, solid lipid nanoparticle; SP, sulfapyridine; SSZ, sulfasalazine; SVCT-1/2, the sodium-dependent vitamin C transporter-1/2; T1D, type 1 diabetes; T1DM, type 1 diabetes mellitus; T2D, type 2 diabetes; TCA, taurocholate; TCDC, taurochenodeoxycholate; TDCA, taurodeoxycholate; the GI tract, the gastrointestinal tract; TLCA, taurolithocholate; TME, the tumor microenvironment; UDC, ursodeoxycholic acid; WHO, World Health Organization.

*Corresponding author.
E-mail address: zggao@imm.ac.cn (Zhonggao Gao).

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Colon-specific drug delivery system

summarized the existing research investigating the possible relationship between the gut microbiota and the bioavailability of oral drugs, which may provide great ideas and useful instructions for the design of novel drug delivery systems or the achievement of personalized medicine.

1. Introduction

The gut microbiota is the collection of bacteria, archaea and eukarya residing the gastrointestinal (GI) tract[1]. It has been currently estimated that the number of the total microorganisms colonising the GI tract is more than 100 trillion[2]. With the development of culture-independent approaches, such as 16S rRNA sequencing and whole-genome shotgun metagenomics[1], it is much easier and more precise to identify the species of the gut microbiota. According to the data from the Human Microbiome Project and the MetaHit, 2172 species of human gut microbiota have been identified and they have been sorted into 12 distinct phyla. The majority of the species belong to five phyla: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes and Fusobacteria. Bacteroidetes and Firmicutes are the main phyla of the human adult's gut microbiota[3]. The density and variety of human gut microbiota rises distally along the gut[4]. Only fast-growing, facultative anaerobes which can adhere to epithelia or mucus are supposed to reside in the small intestine due to the limitations like a short transit time, lower pH, higher antimicrobial concentration and high levels of oxygen. The ileum and colon are the sites where the most plentiful gut microbiota exists[5,6]. The predominant families of the gut microbiota in the colon are Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae and Ruminococcaceae[7]. The gut microbiota can benefit the host in many aspects. Not only can it harvest energy, produce vitamins and modulate the metabolism of bile acids but also it can protect the host from pathogens, modulate host's immune system and maintain the integrity of the intestinal barrier[1,4,6]. Diet, delivery mode, ethnic origin, age and the use of antibiotics are some of the common factors which can affect the composition of the gut microbiota[8–12] and the dysbiosis of it may induce diseases of the immune system, the endocrine system, the cardiovascular system and the nervous system[13,14].

Oral administration is the most common approach to drug delivery due to its safety, convenience, low cost, a greater degree of flexibility and better patient compliance. It is also a preferred route to drug administration for the treatment of chronic diseases that demand long-term drug administration[15,16]. Good absorption and high bioavailability are very important for the therapeutic efficacy of oral drugs. About 40% of candidate drugs are abandoned because of adverse pharmacokinetics and bioavailability in 1991[17]. The efficiency of this process is subject to drug's physicochemical properties, like drug solubility[18] and permeability[19], individual physiological characteristics, like gastrointestinal pH, gastrointestinal transit time[20], transport systems[21] and other factors like diet[22]. As symbiotic microorganisms in the gut, the gut microbiota has a good chance of contacting with oral drugs, especially those absorbed in the lower gastrointestinal tract. Therefore, the influence of the gut microbiota on the pharmacokinetics of oral drugs could not be neglected. Some research has shown that gut microorganisms can affect oral drug's bioavailability either direct or in indirect ways, such as changing the intestinal properties. Gut microbial enzyme activity can directly influence the bioavailability of oral drugs by affecting their metabolism[23], first-pass effect[24] or enterohepatic recirculation[25]. It may also influence drug pharmacokinetics by participating in the biotransformation of bile acids which can affect the bioavailability of lipophilic drugs[4]. Early research has demonstrated that the gut microbiota can influence the drug transport by substrate competition or regulating the expression of transporters[26,27]. Apart from indigenous intestinal flora, the infection of *Helicobacter pylori*[28] and the supplements of probiotics[29] can also have an influence on this process. Therefore, it is necessary to investigate the association between the gut microbiota and the bioavailability of oral drugs to ensure a better clinical outcome.

Having a deep insight into the relationship between gut microorganisms and oral drugs' bioavailability can help us to have better comprehension of the inter-individual variation in bioavailability, and then providing personalized and precise clinical advice. It may also help us to avoid some potential drug−drug interactions induced by the change in the gut microbiota. Besides, it can also help us to understand the differences of the results obtained from *in vitro* and *in vivo* experiments or the possible different outcomes of oral drug's bioavailability in animal experiments and clinical trials. In addition, it may also offer us many innovative ideas of the drug delivery system design, especially the design of colon-targeted drug delivery systems. Herein, we provide a review to document the recent discoveries of the possible interactions of the gut microbiota, host and oral drugs by which the bioavailability may be affected (as shown in Table 1[24–26,28,30–52]) and make suggestions for further research in this field.

2. The gut microbial enzyme activity

The gut microbiota can secrete diverse kinds of enzymes and therefore it is engaged in many types of biotransformation, such as azo reduction, nitro reduction, sulfoxide reduction, *N*-oxide reduction, hydrolysis, dehydroxylation, decarboxylation, deacetylation and acetylation[53,54]. Unlike the main types of biotransformation in the liver, the oxidation and conjugation, the enzymes of gut microorganisms are mainly involved in the reduction and deconjugation[5,55]. After the oral drugs are administered, they will experience the first-pass effect and the enterohepatic recirculation in the gut and liver, which can affect the pharmacokinetics of these drugs. The gut microbial enzyme activity may be involved in these

Table 1 The influence of gut microbiota on bioavailability of oral drugs.

Possible mechanism	Name	Therapeutic application	The influence on PK or pharmacological effects of the drug	Ref.
Prodrugs are metabolized by the gut microbiota	Prontosil	Antibacterial drug	Activate the drug and the bioavailability is decreased when pretreated with antibiotics	30, 31
	Sulfasalazine	Antibacterial drug	Activate the drug and the bioavailability is decreased when pretreated with antibiotics	32
	Lovastatin	Lipid-lowering drug	The bioavailability and pharmacological effects are decreased when antibiotics are used	33
The gut microbiota participates in its first-pass effect	Deleobuvir	Antiviral drug	Plasma exposure of its major metabolite, CD 6168, is decreased substantially in pseudo-germ free rats	24
	Amlodipine	Cardiovascular drug for hypertension	The systemic exposure of amlodipine is decreased	34
	Nifedipine	Cardiovascular drug for hypertension	The bioavailability of nifedipine is increased when antibiotics are used or in the hypoxic condition	35
	Aspirin	Non-steroidal anti-inflammatory drug	The bioavailability of aspirin is increased when antibiotics are used or after the quick ascent to the plateau	36–38
Microbial β-glucuronidases participate in the enterohepatic recirculation	Mycophenolic acid	Immunosuppressant	$AUC_{0-12\ h}$ of the mycophenolic acid is decreased while CL of it is increased when pretreated with antibiotics	39
	Indomethacin	Non-steroidal anti-inflammatory drug	The half-time is shortened and drug's systemic exposure is reduced when pretreated with antibiotics	25
The drug transport is affected by the modulations of bile acids induced by the gut microbiota	Simvastatin	Lipid-lowering drug	Enhanced plasma concentration of simvastatin is positively correlated with increased levels of several secondary bile acids	40
	Lovastatin	Lipid-lowering drug	NaDC containing formulations have better bioavailability of lovastatin than NaGC containing solid dispersions	41
The expression of drug transporters is affected by the gut microbiota or the compounds derived from the gut microbiota	Ascorbic acid	Antioxidant	The absorption of ascorbic acid is decreased by LPS generated from the gut microbiota	26
	Glycyrrhizic acid	Liver protective drug	The bioavailability of glycyrrhizic acid is increased by the supplements of *Lactobacillus murinus*	42
Eggerthella lenta possessing *cgr* operon converts digoxin into dihydrodigoxin	Digoxin	Cardiovascular drug for heart failure and atrial fibrillation	Inactivate digoxin	43–47
Berberine is converted into dihydroberberine by the gut microbial nitroreductases	Berberine	Treatment for diarrhea, hyperlipidemia and type 2 diabetes	The absorption of berberine is facilitated	48
The infection of *Helicobacter pylori* However, the exact mechanisms remain unknown	Levodopa	Treatment for Parkinson's disease	The absorption and therapeutic efficacy of levodopa are impaired by the *Helicobacter pylori* infection	28,49–51
The state of hepatic sulphate can be influenced by the gut microbiota	Acetaminophen	Non-steroidal anti-inflammatory drug	The AUC of acetaminophen is higher in pseudo germ-free rats compared with control rats	52

AUC, the area under the curve; CL, clearance; LPS, lipopolysaccharide; NaDC, sodium deoxycholate; NaGC, sodium glycholate; PK, pharmacokinetics.

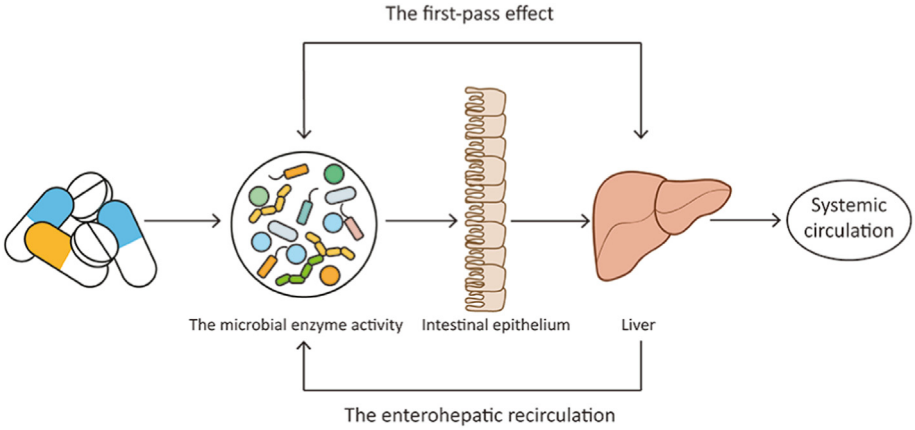

Figure 1 The effects of the gut microbial enzyme activity on the first-pass effect and the enterohepatic recirculation. After being administered orally, some of the drugs can be metabolized by microbial enzymes before absorption. Then the drugs and metabolites can be transported to the liver *via* the portal vein. In the liver, some of the drugs may go through the oxidation and conjugation caused by hepatic enzymes. After that, drugs and/or their metabolites can enter the systemic circulation or be delivered back to the intestine where they can be reactivated by some microbial enzymes or enzymes in the gut and transported to the liver again. Both the first-pass effect and the enterohepatic recirculation can influence the bioavailability of oral drugs.

processes and thereby may influence the bioavailability of oral drugs (Fig. 1). Besides, the gut microbiota may also affect the absorption of prodrugs and influence the oral drugs' bioavailability by modulating the metabolism of bile acids.

2.1. *The influence of the gut microbial enzyme activity on prodrugs*

The prodrug is an inert derivative of an original drug molecule which can release pharmacologically active drug through the enzyme or non-enzyme conversion *in vivo*[56]. Since biotransformation plays a critical part in liberating the biologically active moiety, the efficacy of prodrugs can be influenced by the gut microbial enzyme activity.

As azo reduction can be conducted by the gut microbiota in the colon, conjugating the active moiety with a carrier by the azo bond is a strategy for prodrug design. It has been reported that the gut microbiota can be engaged in azo reduction since the 1960s[57,58]. The major anaerobic bacteria which can excrete azoreductases belong to the genera *Clostridium* and *Eubacterium*[59]. Some probiotic strains like *Lactobacillus acidophilus*, *Bifidobacterium lactis* and *Streptococcus salivarius* are also capable of reducing azo compound[60]. Azo bond is cleaved by azoreductases through a ping-pong mechanism by which NADH acts as an electron donor *via* a hydrazine intermediate[61]. One of the typical azo prodrugs is prontosil. The therapeutic active part of prontosil is sulphanilamide which is derived from it by azoreductases[62]. In addition to the conversion by liver and kidney, prontosil can be split into sulphanilamide by intestinal flora, which can be suppressed by antibiotics in rats[30]. Another kind of azo prodrug is sulfasalazine (SSZ) which was introduced into clinical practice by Svartz[63] in 1941 and now is widely used to treat patients for inflammatory bowel disease (IBD) and rheumatoid arthritis[64]. After oral administration, only 12% of sulfasalazine is absorbed in the stomach and small intestine[56], the majority of it is reduced by the gut microbiota to release 5-aminosalicylic acid (5-ASA) which is pharmacologically active. For SSZ which is absorbed in the upper gastrointestinal tract, it will partly be taken up by the liver and excreted in the bile back into the gut where it will be biotransformed by gut flora[65]. This was supported by the experiment in germ-free and conventional rats[32,65]. Since it is a colonic disease that is treated by 5-ASA clinically, it is better to deliver 5-ASA directly to the diseased site. However instead of reaching the colon, free 5-ASA is absorbed in the upper small intestine after oral administration[66]. So it is a great idea to conjugate 5-ASA to a moiety which acts as a carrier to target it specifically to colon[23]. As side effects of SSZ are mainly ascribed to the release of sulfapyridine, other safer molecules are used to replace it to produce the analogues of SSZ. Such prodrugs are olsalazine, ipsalazide and balsalazide[66,67]. Apart from the prodrugs mentioned above, the bioavailability of lovastatin, a kind of lipid-lowering drugs, may also be influenced by the gut microbiota. Research showed that the systemic exposure to one of the metabolites of lovastatin decreased significantly in the rats pretreated with antibiotics compared with controls[33].

To sum up, the enzyme activity of the gut microbiota plays a crucial role in the biotransformation of prodrugs, offering us many good ideas to design colon-targeted drug delivery systems which we will discuss in Section 7.

2.2. *The influence of the gut microbial enzyme activity on the first-pass effect of oral drugs*

After the drug is taken orally, it may be metabolized in the gastrointestinal tract or liver before it is exposed to the systemic circulation. This process is termed the first-pass effect and it influences the bioavailability of oral drugs[68]. The first-pass metabolism of the gut microbiota may influence the pharmacokinetics of oral drugs, and therefore it may impact their pharmacological efficacy[69]. The rate and extent of gut microbial biotransformation can be affected by the quantity of drugs reaching the lower gastrointestinal tract[70]. As the structural complexity of newly designed drugs is increasing, their solubility and permeability may be impaired. So these newly developed drugs tend to belong to biopharmaceutics classification system (BCS) class II drugs or BCS class III or IV drugs, resulting in longer residence in the gut

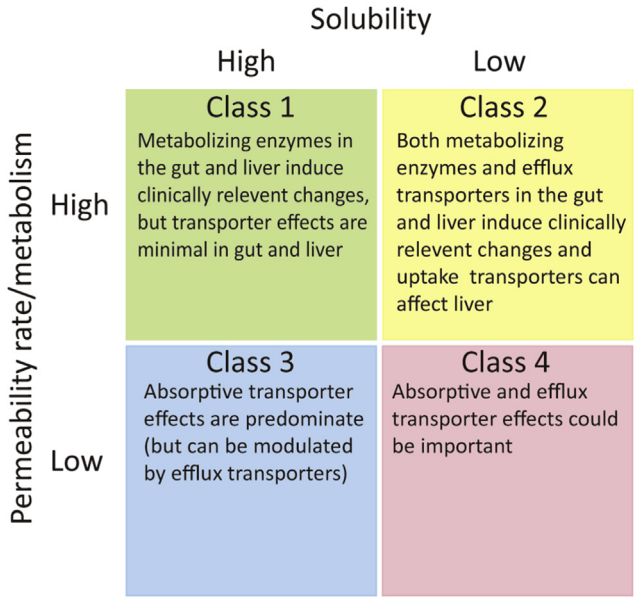

Figure 2 Four biopharmaceutics drug disposition classification system (BDDCS) classifications of oral drugs and the prediction of the metabolizing enzyme and transporter effects by BDDCS[74].

and higher drug concentrations in the colon[71]. In addition, with the development of modified release preparations like colon-specific or extended release systems, the investigation of the influence on such oral drugs' bioavailability by gut microbial metabolism is of increasing importance[72]. Meanwhile, we also need to pay attention to drugs belonging to the biopharmaceutics drug disposition classification system (BDDCS) class 1 and BDDCS class 2. The BDDCS was first introduced by Wu and Benet in 2005[73]. In BDDCS, the extent of metabolism is substituted for the extent of permeability in BCS while the same criteria as the FDA for high and low solubility are still remained[74]. Predictions in BDDCS are based on intestinal permeability rate since a strong relationship between it and the extent of metabolism exists. Compared with BCS, BDDCS is not affected by transporter effects and can get rid of the situation in which drugs are sorted into more than one class as the permeability measures in BCS from various studies are uncertain[74]. BDDCS can be used to predict the disposition of new molecular entities (NMEs) and possible drug−drug interactions for NMEs and drugs on the market relating to the intestine and liver[74,75]. The drugs are divided into four classifications based on the extent of metabolism and the extent of solubility, and the effects of metabolizing enzymes and transporters predicted by BDDCS following oral dosing are shown in Fig. 2. For class 1 drugs with high metabolism and high solubility, metabolizing enzymes in the intestine and liver play an important role in their disposition while transporters' effects are minimal in the gut and liver. For class 2 drugs with high metabolism and low solubility, apart from the effects of metabolizing enzymes, efflux transporters in the gut and both uptake and efflux transporters in the liver can have an influence on their disposition. Metabolizing enzymes are unlikely to influence the disposition of class 3 and class 4 drugs while the effects of uptake or efflux transporters in the gut and liver play a predominate role[74,75]. Since the pharmacokinetic characteristics of BDDCS class 1 drugs and BDDCS class 2 drugs can be clinically affected by metabolizing enzymes, whether the gut microbial enzyme activity can influence their bioavailability *via* pre-uptake metabolism should also be investigated.

It has been reported that gut microbial enzymes converted deleobuvir, an antiviral drug, into its major circulating metabolite, CD6168, before systemic absorption. CD6168 plasma exposure was about 9-fold lower in pseudo-germ free rats than that in control rats[24]. Besides, the gut microbial enzyme activity is also involved in the first-pass effect of some cardiovascular drugs. Yoo et al.[34] demonstrated that the gut microbiota was involved in the metabolism of amlodipine, a BDDCS class 1 drug[74] used for hypertension. The bioavailability of amlodipine was found to be increased in rats pretreated with ampicillin, which might strengthen the therapeutic efficacy of amlodipine. This research also indicated that there might be a potential drug−drug interaction between amlodipine and ampicillin in the clinical treatment. Zhang et al.[35] demonstrated that the gut microbial enzyme activity was involved in the metabolism of nifedipine, another antihypertensive drug belonging to BDDCS class 2[74]. Interestingly, they also found that both antibiotics and the plateau hypoxia could attenuate the metabolism of nifedipine and enhance their bioavailability by inducing the alterations in the gut microbiota, which indicated that the clinical dose of nifedipine might need to be adjusted at high altitude or when the antibiotics are co-administered.

The first-pass metabolism of aspirin, a BDDCS class 1 drug[76] belonging to non-steroidal anti-inflammatory drugs (NSAIDs), also involved the enzyme activity of microbiota. It was generally considered that upper gastrointestinal tract was the main site where aspirin was absorbed, so the influence of the gut microbiota on the absorption or metabolism of aspirin would be neglected. However, Kim et al.[36] proved that the gut microbiota was responsible for the biotransformation of aspirin to its major metabolite, salicylic acid. They also reported that antibiotics could increase the absorption of aspirin and potentiate its antithrombotic effect by suppressing the metabolic activity of gut bacteria. They also found that the plasma level of salicylic acid was increased, but it was mainly due to the conversion of plasma aspirin (the absorbed aspirin) than that in the intestine. This is consistent with the results of another study conducted by Zhang et al[37]. The reason why the gut microbiota can affect the biotransformation of aspirin before absorption may be that gut microorganisms are not only limited to the large intestine. The gut microbiota also distributes and reacts in the upper gastrointestinal tract though the amount of it may not as much as that in the colon. Besides, in the clinical application, the enteric coated aspirin is administered to the patients to avoid the absorption in the stomach. So the influence of the gut microbiota on aspirin's absorption in the clinical treatment might be greater than those observed in the research[36]. The pharmacokinetics and pharmacodynamics of aspirin can also be influenced by the change in the gut microbiota induced by hypobaric hypoxia as nifedipine mentioned above. Sun et al.[38] found that a significant increase in *Bacteroides* in rat feces was caused by the plateau hypoxic environment, while *Corynebacterium*, *Prevotella*, and *Coprococcus* were decreased. These alterations induced increasing aspirin's absorption in the rats after the quick ascent to the plateau and the use of aspirin was found more possible to induce bleeding under this condition. Therefore, it might be recommended that the aspirin dose should be reduced for the people who rapidly enter the plateau.

As we can conclude from the research mentioned above, gut bacteria can influence the bioavailability of oral drugs by being involved in the first-pass effect. The factors which can alter the

enzyme activity of the gut microbiota may influence the pharmacological potency of these drugs. The most common factor is the use of antibiotics, so it is necessary to investigate the interaction between antibiotics and the oral drugs which undergo the first-pass metabolism in the gut. As there are differences between the actual composition of gut microorganisms of animal models and those of humans, it is hard to extrapolate from the experiments conducted with animals. Therefore, clinical trials and better models or methods which can simulate the real composition and distribution of human gut microbiota are needed. In addition, the influence of dosage forms used in the clinical treatment also needs to be taken into consideration when we study the gut microbial biotransformation or drug−drug interaction of these drugs.

Since the influence of the first-pass effect induced by the gut microbiota is realized, there are several strategies which may be helpful to elevate the oral drugs' bioavailability based on the findings above. First, we may improve the solubility or permeability of some drugs to reduce their residual time in the gut and decrease their colonic concentration. Poor solubility can be improved by the methods such as micronization, formulating drugs as solid dispersions or the cyclodextrin inclusion complex, and the application of the nanoformulation[18], while the permeability can be enhanced by strategies such as modifying the chemical structure of the drug, using permeation enhancers, ion-pairing method and diverse nanotechnology-based methods[19]. Then it has been reported that some kinds of mucopenetrating particles and the conjugation of enzyme inhibitors can be used to avoid the intestinal pre-uptake metabolism. In addition, some complexing agents like EDTA and diverse surfactants or co-surfactants which are used to design lipid-based carriers are found to be able to inhibit metabolizing enzymes in the gut[77]. These approaches applied to preventing the drug degradation caused by enzymes may offer us some useful ideas to lower the effects of the pre-uptake metabolism induced by the gut microbiota and increase the oral drugs' bioavailability when we design novel drug delivery system.

2.3. *The influence of microbial β-glucuronidases on the enterohepatic recirculation of oral drugs*

Apart from first-pass effect, the enterohepatic recirculation can also have an influence on the bioavailability of oral drugs. After being absorbed from the gastrointestinal tract, the drugs are metabolized in the liver to become less toxic and more hydrophilic. Some of the metabolites can be delivered back to the GI lumen *via* bile duct and be transported to the liver again after being reactivated and absorbed *via* the intestinal epithelia. This process is termed enterohepatic recirculation[78]. Gut microbial β-glucuronidases are crucial in this process. β-glucuronidases can catalyze the hydrolysis of the glucuronidated drugs which are formed in the liver by uridine diphosphate (UDP)-glucuronosyltransferases to liberate glucuronic acid as a carbon source, effectively releasing and reactivating the aglycones in the GI tract[78,79]. There are many gut microbiota genera that can secrete β-glucuronidases, like *Clostridium*, *Peptostreptococcus*, and *Staphylococcus*[80]. The alteration in the β-glucuronidase activity may affect the pharmacokinetics of oral drugs which undergo the enterohepatic recirculation. AUC_{0-12h} of mycophenolic acid (MPA), a kind of immunosuppressants, was reduced to 75%−80% and CL_{tot} of MPA was increased about 1.4−1.8-fold due to the inhibition of the β-glucuronidase activity by amoxicillin/clavulanate[39]. For indomethacin, a kind of NSAIDs, pretreating mice with antibiotic mixtures induced less drug exposure and a shorter half-life of it, which might be attributed to the reduced bacterial β-glucuronidase activity[25].

In addition to the alteration in pharmacokinetics, the toxicity induced by the hydrolysis of β-glucuronidases receives much attention recently. The most typical example is irinotecan whose metabolite SN-38 liberated by β-glucuronidases induces severe diarrhea in cancer patients[79,81]. Locally high concentrations of NSAID aglycones released by β-glucuronidases can also cause damage to the distal small intestine by the mechanisms of endoplasmic reticulum stress and mitochondrial stress[82]. To solve this problem, different kinds of β-glucuronidase inhibitors are developed and here is a comprehensive review recommended about this topic[83].

2.4. *The influence of microbial bile salt hydrolase (BSH) enzymes and microbial 7α-dehydroxylases on bile acid metabolism*

The gut microbiota can also affect the bioavailability of oral drugs by modulating the metabolism of bile acids. Bile acids are amphipathic molecules characterized by a large, rigid, and planar hydrophobic steroid nucleus with variability in number, position and stereochemistry of hydroxyl groups, along with a flexible acidic side chain[4,84,85]. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are primary bile acids which are synthesized in hepatocytes from cholesterol. Then they are conjugated to either taurine (approximately 25%) or glycine (approximately 75%) in the liver. Since the conjugation respectively decreases the dissociation constant (pK_a) of bile acids from around 5[86] to about 4[86] and 2[87] in the case of glycine- and taurine-conjugates and the pH range of intestinal is about 6.0−7.5, these conjugates exist in an ionized form as bile salts[4]. Bile salts are physiological surfactants. The concave (α) side of the steroid skeleton of bile acid molecules is hydrophilic because of the existence of OH groups, while the convex (β) side possessing angular methyl groups is hydrophobic[88]. Bile salts can increase the absorption of drugs by diverse mechanisms such as forming micelles, interacting with biological membranes and inhibiting the active efflux of P-glycoprotein (P-gp) substrates[88,89]. Therefore, they are always used as an effective absorption enhancer in the drug delivery system's development[90−92].

Conjugated primary bile acids can be biotransformed into secondary bile acids by the gut microbiota in the intestine as shown in Fig. 3. Firstly, the deconjugation of taurine/glycine conjugates is catalyzed by microbial bile salt hydrolase (BSH) enzymes, liberating free primary bile acids CA and CDCA. Secondly, these free primary bile acids serve as substrates of 7α-dehydroxylation conducted by microbial 7α-dehydroxylases, forming deoxycholic acid (DCA) from CA and lithocholic acid (LCA) from CDCA. As the existence of enterohepatic recirculation, both primary and secondary bile acids can be conjugated to taurine/glycine again in the liver. Thus, unconjugated and conjugated forms of both primary and secondary bile acids exist in the human bile acid pool. The gut microbial metabolism of bile acids is mainly due to the activity of BSHs and 7α-dehydroxylases. BSHs are in the Ntn hydrolase superfamily of enzymes[93] and have been isolated from many species of the gut microbiota like *Bifidobacterium bifidum*[94] and *Lactobacillus rhamnosus*[95]. Deconjugation of bile acids can benefit the gut microbiota for it may be related to the detoxification of conjugated bile salts[96] and the acquisition of carbon, nitrogen and sulfur which are essential for its growth[97]. Unlike BSHs, 7α-dehydroxylases are only found in about 0.0001% of total intestinal bacteria[98] and they

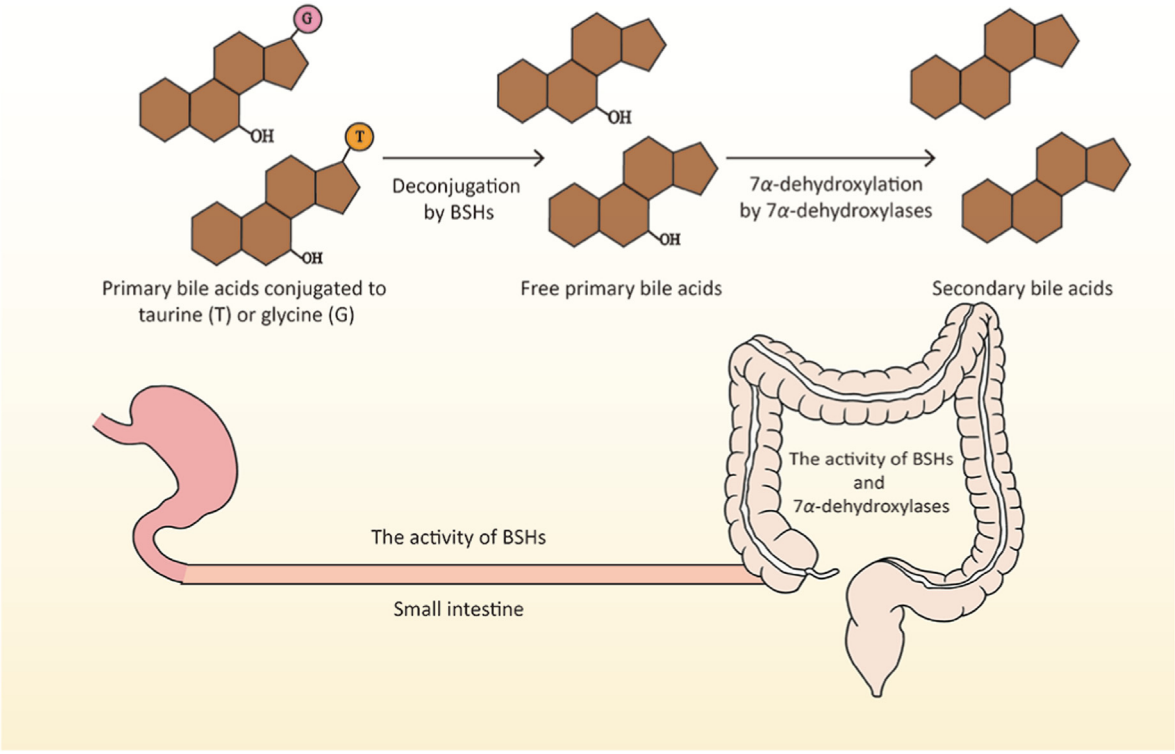

Figure 3 The metabolism of bile acids modulated by BSHs and 7α-dehydroxylases. The conjugated primary bile acids are released in the duodenum to facilitate the digestion and then can be converted to free primary bile acids, CA and CDCA, by BSHs in the terminal ileum and colon. These unconjugated primary bile acids are then biotransformed into secondary bile acids after 7α-dehydroxylation by 7α-dehydroxylases in the colon. CA, cholic acid; CDCA, chenodeoxycholic acid.

are encoded by members of the Firmicutes phylum such as *Clostridium* cluster XVIa[4]. The deconjugation conducted by BSHs is the prerequisite to 7α-dehydroxylation which may involve two steps proposed by Samuelsson: diaxial trans-elimination of the 7α-hydroxy group and 6β-hydrogen atom, followed by reduction through trans-hydrogenation of the 6β and 7α positions of the cholen-6-oic acid intermediate forming DCA[99]. However, many types of research indicated that the actual mechanism for 7α-dehydroxylation was more complex than Samuelsson's assumption[98,100,101].

The alteration in human bile acid pool's composition induced by the change in the gut microbial enzyme activity may influence the absorption and bioavailability of oral drugs. Although there has been a paucity of research in this field up to now, the mechanisms can be concluded as follows according to the work we have found.

Firstly, the micellization ability for poorly water-soluble drugs (PWSDs) may be affected by the activity of gut microbial 7α-dehydroxylases. It has been reported that the bile salts' micellization capacity is associated with the number and orientation of hydroxyl groups attached to the steroid backbone. Generally, the addition of hydroxyl groups[102−104] and alteration in a hydroxyl group from α-to β-configuration[85,105] can increase the CMC values. Since the unconjugated primary bile acids can be converted into secondary bile acids *via* 7α-dehydroxylation by the gut microbiota, the ability of bile acids to solubilize drugs may be changed. Enright et al.[106] found that the solubilization capacity of sodium taurodeoxycholate (TDCA, a kind of secondary bile acids) micelles was higher than that of sodium taurocholate (TCA, a kind of primary bile acids) micelles for all nine PWSDs studied *in vitro*, indicating that the gut microbiota may affect the drug absorption process by gut microbial 7α-dehydroxylation (Fig. 4). However, the effect showed in this experiment is drug-specific and further *in vivo* experiments are still needed to support this hypothesis.

Secondly, the gut microbial metabolism of bile acids may influence the absorption by affecting the time of crystallization inhibition. Bile salts potentially inhibit crystallization to maintain supersaturation, a state in which the drug solubility is generally much higher than that of the crystalline form. So it is important to prevent nucleation for enough time for drug absorption[107]. It has been demonstrated that unconjugated bile salts tend to be better at prolonging induction time compared with conjugated bile salts which are more hydrophilic. The inhibition ability is related to the hydrophobicity of bile salts and the structure of drugs. For unconjugated bile salts, the higher the hydrophobicity is, the better the nucleation inhibition properties are. For bile salts conjugated to glycine in this experiment, the contrary trend was observed[108].

Thirdly, bile acid modifications may affect drug transport by inhibiting drug transporters or competing with drug molecules as substrates. Some bile salts like taurolithocholate (TLCA), taurochenodeoxycholate (TCDC), glycochenodeoxycholate (GCDC) and ursodeoxycholic acid (UDC) can inhibit active efflux of P-glycoprotein (P-gp) substrates probably by altering the environment of P-gp or by the interaction with P-gp. The common structural characteristics of these bile salts are the absence of a hydroxyl group at position 12, which may be the reason for P-gp inhibition[89]. Enright et al.[109] reported that the expression of genes encoding human drug transporters could be changed by both free/dihydroxy bile acids. Besides, microbial BSHs might improve multidrug

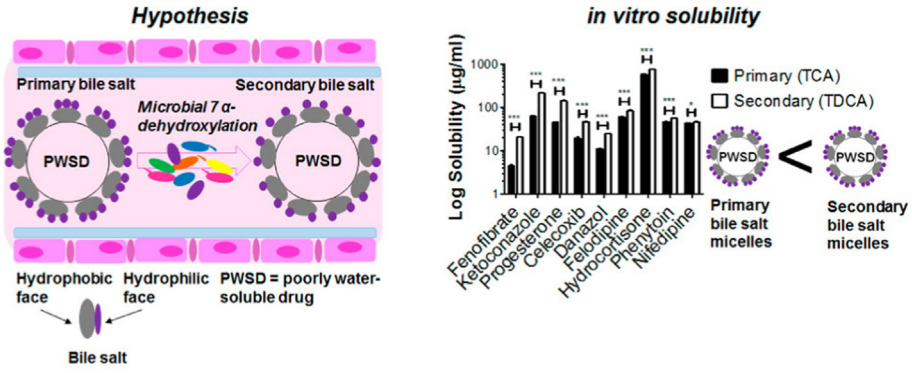

Figure 4 Schematic illustration of the effects of the gut microbial 7α-dehydroxylase activity on the solubilization capacity of bile salt micelles for poorly water-soluble drugs investigated *in vitro*. Reprinted with the permission from Ref.107. Copyright © 2017 American Chemical Society.

resistance by generating deconjugated bile acids which possess the ability to access and inhibit P-gp ATPase (Fig. 5). In addition, the modifications of bile acids may also influence the bioavailability of statins, a kind of lipid-lowering drugs, by influencing the drug transport. In the liver and intestine, bile acids and statins share the same transporters: multidrug resistance gene 1 (*MDR1*, *ABCB1*) P-glycoprotein, multidrug resistance-associated protein 2 (MRP2, ABCC2), and organic anion-transporting polypeptide 1B1[110,111]. Researchers reported that the transport of simvastatin may be influenced by bile acids, which may affect its therapeutic efficacy[40]. A positive correlation between the increased plasma concentration of simvastatin and higher levels of several secondary bile acids has been observed[40]. So there is a hypothesis that primary bile acids may compete with simvastatin for the same transporters and the biotransformation induced by microbial enzymes may reduce this competition, leading to better clinical therapy[69,79]. However, the actual mechanism(s) of the relationship between bile acids and the efficacy of statins remains unknown. Apart from simvastatin, the inhibition of P-gp by bile acids may also contribute to the enhancement of bioavailability of lovastatin and it was found that sodium deoxycholate (NaDC) containing formulations revealed higher bioavailability than sodium glycholate (NaGC) containing solid dispersions of lovastatin[41].

Lastly, the absorption of oral drugs can be affected due to the formation of ion pair complexation and the change in distribution coefficient. Since bile salts are anionic in aqueous solution at physiological pH, they can form ion pair complexation with cationic drug molecules to enhance the absorption. Bile salts can also incorporate into cellular lipid bilayers thus increasing negative charge of the membrane surface, which potentially changes the partitioning of ionized drugs[4]. Yang et al.[112] reported that the apparent distribution coefficient of propranolol in a liposome/buffer system was enhanced by bile salts to various extent [deoxycholate (DC)>cholate(C)>taurocholate (TC)]. The predominant mechanism might be the insertion of bile acids into liposome membranes resulting in more surface negative charges, thus providing stronger electrostatic interactions with the cationic propranolol molecules.

However, it is noteworthy that many effects observed in the experiments mentioned above are drug-specific, so it is hard to extrapolate general trends from these results. Besides, the bile acid metabolism and the number and types of gut microbial enzymes in animal models may be different from those in humans. For example, the 26-hydroxylase has little activity compared with the mitochondrial 27-hydroxylase in humans while the microsomal 26-hydroxylase may play an important role in the rat[113]. As most of the experiments in this field are conducted *in vitro*, it is hard to predict the actual influence of gut microorganisms on the bioavailability of oral drugs *via* the bile acids modification in human bodies. Therefore, further *in vivo* experiments and clinical trials are still required to prove these conclusions.

3. The alteration in the drug transport

The process by which drugs pass through biological membranes is termed transport. This process is important to the bioavailability of oral drugs. According to the mechanisms, it can be generally divided into three types: passive transport, carrier transport and cytosis transport. In the process of passive transport, drugs go through biological membranes without the involvement of membrane transport proteins. There are two kinds of passive transport. One is filtration through pores by which water-soluble molecules pass through biological membranes *via* pore structure with a large number of water molecules. The other one is passive diffusion by which drugs diffuse from the higher concentration to the lower concentration. This is also the transport mechanism for most kinds of drugs. As for carrier transport, it can be divided into active transport and passive facilitated diffusion. Drugs can be transported against a concentration gradient with the consumption of ATP *via* active transport while the direction of transport is determined by the solute concentration gradient or electrochemical gradient without an energy input when drugs are transported *via* passive facilitated diffusion. Most of the membrane transport proteins found in intestinal tissues belong to two major classes of transporters, the ATP-binding cassette (ABC) and solute carrier (SLC) family[114]. Eukaryotic ABC transporters are exclusively efflux transporters and the representatives are multidrug resistance protein (P-gp, MDR1) and breast cancer resistance protein (BCRP)[115]. On the contrary, apart from multidrug and toxin extrusion proteins (MATEs), SLC transporters largely function as uptake transporters which play a critical part in the uptake of small molecules into cells[116]. Organic cation transporters (OCTs), organic zwitterion/cation transporters (OCTNs) and organic anion transporters (OATs) all belong to this class[117,118]. The factors which can influence the expression and function of these intestinal transporters may affect the

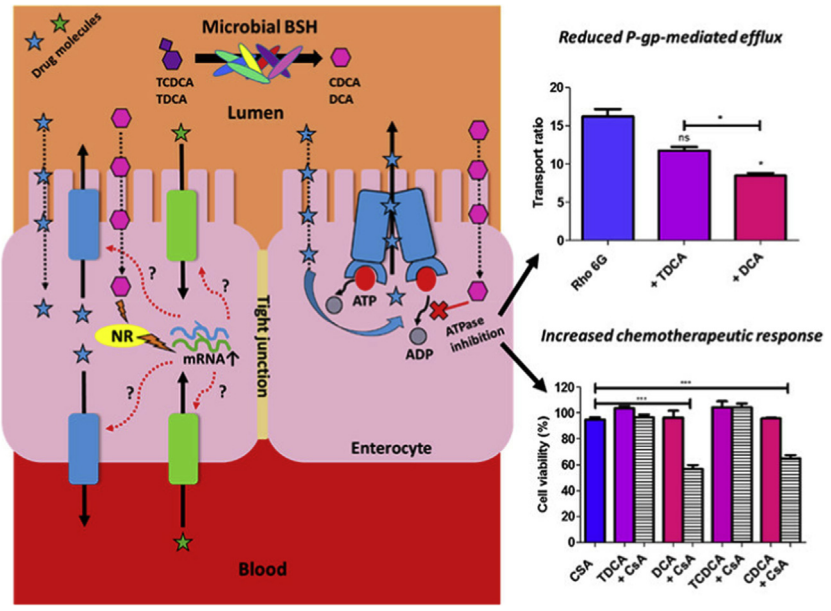

Figure 5 Schematic illustration of the effects of deconjugated bile acids generated by BSHs on the inhibition of the P-gp ATPase. Reprinted with the permission from Ref.110. Copyright © 2018 American Chemical Society.

bioavailability of oral drugs. Unlike small molecules, macromolecules like protein, nucleic acid and polysaccharide are transported through biological membranes by cytosis transport which is achieved by the formation and the fusion of vesicles.

The gut microbiota may influence the bioavailability of oral drugs by affecting the process of transport. It has been reported that passive absorption is prone to be increased without the presence of microbiota[119]. According to the research we have collected, the gut microbiota may affect the bioavailability of oral drugs or nutrients in the following two ways.

Firstly, the gut microbiota or their metabolites may influence the transport process by regulating the expression of host genes. One example is the influence on the expression of sodium-coupled glucose transporter 1 (SGLT-1), a Na^+/glucose cotransporter. For ileal levels of *Sglt*-1 mRNA, it increased after the colonization of *Bacteroides thetaiotaomicron* in the germ-free mice[120]. Cresci et al.[121] reported that SGLT-1 was also expressed on the apical membrane of colonic epithelial cells to help the absorption of glucose with the promotion of Na^+ absorption in the colon and its expression was silenced in the germ-free state. However, for the expression of SGLT-1 which existed in the intestinal epithelium, it was significantly enhanced in the germ-free mice compared with controls. The reason might be that the absence of gut microorganisms resulted in a compensatory mechanism which allowed the proximal intestine to detect and absorb nutrients by the regulation of transporters' expression to overcome the deficits of nutrients absorption in distal GI tract in germ-free state[122]. Apart from SGLT-1, the expression of transporters such as multidrug resistance protein-1a (MDR1a), SLC5A8, SLC26A3 and aquaporin 4 (AQP4) is also affected by the gut microbiota. MDR1a was found fourfold decreased after colonizing GF mice with *B. thetaiotaomicron*[120] while the expression of butyrate transporter SLC5A8, chloride/bicarbonate exchanger SLC26A3 and AQP4 involved in water absorption was silenced in GF mice[121]. Yuan et al.[42] reported that *Lactobacillus murinus* could enhance the bioavailability of oral administered glycyrrhizic acid (GL) by significantly downregulating the efflux transporter gene expression level of multidrug resistance gene 1 (*MDR1*) and multidrug resistance protein 2 (MRP2) *in vitro*. They also found that the supplements of *L. murinus* could prominently increase the bioavailability of GL in rats with liver cirrhosis, indicating a potential strategy for enhancing the efficacy of liver protective drugs in the clinical treatment. Another representative example is the inhibition of intestinal ascorbic acid (AA) uptake by lipopolysaccharide generated by the gut microbiota. Lipopolysaccharide (LPS) can be generated by the gut microbiota and pathogens like *Salmonella* and the levels of it are increased in the blood of patients with some inflammatory diseases like IBD, necrotizing enterocolitis (NEC) and *Salmonella* infection. It was found that LPS down regulated the expression of *SLC23A1* and *SLC23A2* transcription *via* the inhibition of the level of expression and function of both HNF1α and Sp1 transcription factors which were essential for basal *SLC23A1* and *SLC23A2* promoter activity. As the reduced ascorbic acid form was transported by the sodium-dependent vitamin C transporter-1 and -2 (SVCT-1 and SVCT-2) which were respectively the products of *SLC23A1* and *SLC23A2* genes, the carrier-mediated uptake of ascorbic acid was inhibited with the presence of LPS[26]. Intriguingly, LPS could be detected in the plasma of the patients with sepsis and there was research showing that some relationship between sepsis and depletion of AA existed. Additionally, it was demonstrated that the use of intravenous vitamin C in an early stage, together with moderate-dose hydrocortisone and thiamine, might prove to be the better treatment for septic patients[123]. Therefore, the author hypothesized that LPS might be critical in the deficiency of AA in sepsis *via* inhibiting the AA absorption in the intestine[26].

The other mechanism by which the gut microbiota can affect transport process is the substrate competition[124]. If the compounds

derived from the gut microbiota or produced by the gut microbiota structurally resemble a drug that is actively transported, they can influence its absorption or bioavailability by competing with it while transporting. González-Sarrías et al.[27] reported that urolithin A which was derived from ellagic acid by the gut microbiota was the substrate for the breast cancer resistance protein (ABCG2/BCRP). This finding indicated that urolithin A might affect the pharmacokinetics and bioavailability of the drugs which were also the substrates of this transporter and the influence might result in undesirable side effects. Further *in vivo* research is required to confirm this hypothesis.

4. The influence of probiotics on the bioavailability of oral drugs

According to the definition formulated by FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) working group experts in 2002, probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host"[125]. The common genera of probiotics used in the products are *Lactobacillus*, *Bifidobacterium*, *Lactococus*, *Streptococcus*, *Enterococcus*, *Bacillus* (Gram-positive bacteria) and *Saccharomyces* (yeast)[126]. Until now, many researchers have reported probiotics possess the abilities to modulate the gut microbiota and maintain balance in the ecosystem[127], produce B group vitamins (*e.g.*, *Lactobacillus plantarum*, *Lactobacillus reuteri*)[128], facilitate the absorption like vitamins and mineral compounds, modulate immune system[129] and show efficacy in antibiotic[130] or anti-cancerogenic treatment[131]. Therefore, loads of clinical trials conducted suggesting that probiotics might assist in the prophylaxis and treatment for gastrointestinal diseases[132], allergic diseases[133], obesity[134], type 2 diabetes (T2D)[135], cancer and associated side effects[136], and depressive symptoms[137], etc. As they are widely used in the dietary supplements, functional food and pharmaceutical formulas, increasing attention should be paid to their potential influence on the pharmacokinetics of drugs which may be co-administered. The possible mechanisms of probiotics' influence on the bioavailability of some drugs investigated can be generally listed as follows: (1) the alteration in local intestinal properties like local pH, transient time and the thickness of the adherent mucous; (2) the influence on drug transport; (3) the change in gut microbial activities; (4) the influence on drug's metabolism. Representative oral drugs whose bioavailability can be influenced by probiotics are listed in Table 2.

Amiodarone (AMI) is the drug used in the treatment for ventricular tachycardia and ventricular fibrillation. Matuskova et al.[29] investigated the influence of probiotic, *Escherichia coli* Nissle 1917 (EcN), on the absorption of AMI in rats. After pretreating experimental group with probiotic suspension daily for a week, amiodarone hydrochloride was administered orally as a single dose (50 mg/kg) to all rats. They found that AMI AUC_{0-30h} was increased by 43% in the group pretreated with probiotic *E. coli* strain compared with controls. Intriguingly, this effect could not be observed when the probiotic *E. coli* strain was substituted with the non-pathogenic but non-probiotic *E. coli* strain. One of the possible explanations for the enhanced absorption of AMI is that EcN induced the reduction in local pH in the intestine. As AMI is a weak base which can be better ionized when pH is lower, it is easier for the drug to diffuse through mucus. In addition, the increased expression of the OATP2B1 (SLCO2B1) transporter whose substrate is AMI may also contribute to the effect. TNF-α, a kind of proinflammatory cytokine, can modulate the expression of this drug transporter. According to the literature, EcN decreases the levels of TNF-α, which may in turn increase the expression of OATP2B1 (SLCO2B1) transporter. However, this is only a drug-specific effect from which results of other probiotics or drugs could not be extrapolated directly.

Gliclazide is a sulphonylurea drug usually used to treat patients for T2D; however, it was found to exert a hypoglycemic effect when combined with a kind of primary bile acid in an animal model of type 1 diabetes (T1D)[144,145]. Besides, the extrapancreatic effects of gliclazide may also benefit type 1 diabetes mellitus (T1DM) treatment, which makes it a potential candidate to treat patients suffering from T1DM when it is combined with other hypoglycaemic agents like bile acids and probiotics[146]. Therefore, it needs to explore the influence of probiotics on the pharmacokinetics of gliclazide or bile acids. After pretreating healthy rats and rats with alloxan-induced T1D with probiotics (*L. acidophilus*, *B. lactis*, and *L. rhamnosus*), gliclazide was given to all rats. Researchers found that gliclazide absorption was reduced in healthy group while it was increased in T1D group. They suggested that this effect might be attributed to the probiotics' influence on MRP2 and MRP3 transporters which were responsible for the efflux of gliclazide[138]. This hypothesis was supported by another experiment which investigated the probiotics' influence on gliclazide permeation *in vitro*, suggesting that MRP2 might be upregulated by bacterial metabolites. Since the function of MRP2 was impaired in rats with T1D, the normalization of the functionality of transporters induced by probiotics resulted in a net absorption[139]. Bacterial degradation and a 'thicker' layer of the adherent mucous formed by probiotics might also be the possible mechanism to explain the decreased gliclazide bioavailability in healthy rats[138]. The effect of probiotics on a semisynthetic primary bile acid, monoketocholic acid (MKC) was also investigated when co-administered with gliclazide. It was observed that the bioavailability of MKC decreased after the pretreatment with probiotics in a healthy group while it remained the same in T1D group, which might result from the increased presystemic metabolism of MKC induced by probiotics[140]. Dose-dependent and longer probiotics treatment effects should be investigated respectively in the future. Besides, the possible influence of alloxan used to induce T1D models on MKC pharmacokinetics should be ruled out.

Probiotics can also affect the bioavailability of acetaminophen and amlodipine in mice and the rabbit model respectively. It was observed that the AUC of orally administered acetaminophen decreased to 68.4% after pretreating with *L. reuteri* KCTC3679 which could increase the numbers of clostridia, bifidobacteria, and enterococci in mice. This effect may be induced by the alteration in the activity of microbial drug-metabolizing enzymes caused by probiotics. *L. reuteri* KCTC3679 facilitates the degradation of acetaminophen directly or *via* increasing gut bacterial acetaminophen-degrading enzyme activity and arylsulfate sulfotransferase activity, which may limit the absorption of acetaminophen. *L. reuteri* KCTC3679 can also stimulate the peristaltic movement resulting in a decrease of the bowel transit time, which may affect the absorption of acetaminophen[141]. The plasma concentration of amlodipine increased in rabbits when they were supplied with *L. plantarum* IS-10506. It was found that the level of red blood cell (RBC) and hematocrit (HTC) increased significantly in the *L. plantarum* IS-10506 supplemented group. Higher levels of RBC and HTC induce the enhancement of blood flow

Table 2 The influence of probiotics on bioavailability of oral drugs.

Name	Probiotic	The influence on PK or pharmacological effects of the drug	Possible mechanism	Model	Ref.
Amiodarone (AMI)	*E. coli* strain Nissle 1917 (EcN)	The bioavailability of AMI is increased.	1. Local intestinal pH is decreased. 2. The expression of the OATP2B1 (SLCO2B1) transporter is increased.	Male Wistar rats	29
Gliclazide	*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus rhamnosus*	Gliclazide absorption in healthy rats is reduced while that is enhanced in a type 1 diabetes rat model.	1. MRP2 and MRP3 may be influenced. 2. A 'thicker' layer of the adherent mucous may be formed. 3. Bacterial degradation.	Healthy male Wistar rats and rats with alloxan-induced type 1 diabetes	138,139
Monoketocholic acid (co-administered with gliclazide)	*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus rhamnosus*	The bioavailability of MKC is reduced in healthy rats treated with probiotics while it remains the same in type 1 diabetes rats when taken with gliclazide together.	Presystemic metabolism of monoketocholic acid may be increased by probiotics.	Healthy male Wistar rats and rats with alloxan-induced type 1 diabetes	140
Acetaminophen	*Lactobacillus reuteri* K8	Acetaminophen AUC decreased after the treatment with probiotics.	1. Acetaminophen metabolism is promoted directly or *via* probiotics' modulation of microbial enzyme activity. 2. A decrease of the bowel transit time	Male C57BL/6 mice	141
Amlodipine	*Lactobacillus plantarum* IS-10506 probiotic	Amlodipine plasma concentrations are increased significantly after the treatment with probiotics.	1. The level of red blood cell (RBC) and hematocrit (HTC) is increased. 2. More ATP-binding cassette transporters are provided by probiotics.	Male New Zealand White rabbits	142
Sulfasalazine	*Lactobacillus acidophilus* L10, *Bifidobacterium lactis* B94, *Streptococcus salivarius* K12	1. In animal models, the metabolism of sulfasalazine is affected while the PK of sulfasalazine or sulfapyridine is not influenced. 2. Both metabolism and PK of sulfasalazine and sulfapyridine are not significantly affected in the clinical trial.	1. The transporters are not influenced in animal models. 2. The barrier function of the colonic mucosa to sulfapyridine absorption is increased in animal models. 3. In the clinical trial, higher levels of the probiotic cell-associated enzyme activity to the intestine is not provided by probiotic treatment. 4. In the clinical trial, indigenous microbial azoreductase activity is not enhanced significantly by probiotics.	1. Male Wistar rats 2. Patients with rheumatoid arthritis	60,143

AUC, the area under the curve; MKC, monoketocholic acid; PK, pharmacokinetics.

and an increase of plasma protein, which may facilitate the amlodipine absorption. In addition, since the ABC transporters can either be exporters or importers in prokaryotic cells, more ATP-binding cassette transporters provided by *L. plantarum* IS-10506 may transport amlodipine *via* the intestinal tract increasing the absorption of amlodipine[142]. However, this experiment also has some limitations. A hypertensive rabbit model group and non-surviving *L. plantarum* IS-10506 should be involved in the experiment. They should also investigate whether there is a dose−depend relationship between the amlodipine absorption and the pretreatment with *L. plantarum* IS-10506 or a normal reference lactic acid bacterium[142].

Probiotics may also have no significant influence on the pharmacokinetics of oral drugs in some instances. In the study investigating the probiotics' influence on sulfasalazine (SSZ) in rat, a higher level of the azoreductase (AR) activity was observed and the sulfapyridine (SP) production increased in colon in the rats pretreated with *L. acidophilus* L10, *B. lactis* B94 and *S.*

salivarius K12, which might be due to probiotics' AR activity. However, the pharmacokinetic parameters of SSZ and SP which was a SSZ metabolite that could cause adverse effects were not significantly changed from the control group. This may be because that pretreatment with probiotics dose not influence the transporters whose substrate is SSZ, and the barrier function of the colonic mucosa to the absorption of SP may be increased by probiotics[60]. In the preliminary study investigating the effects of short-time treatment with probiotics on SSZ metabolism in patients with rheumatoid arthritis (RA), no significant alteration in SSZ metabolism was observed, though the strains of probiotics are the same as those which induced a higher level of the AR activity in the animal models as mentioned above. This indicates that short-time treatment with probiotics could not provide notable additional levels of probiotic cell-associated enzyme activity to the intestine or increase the AR activity of indigenous gut microbiota[143]. The metabolism of this difference should be investigated in further research. These two types of research also indicate that the effects of probiotics on the oral drugs may be different in animal models and human bodies, so more clinical trials may be in need to instruct the clinical use of probiotics.

5. The influence on special drugs

Apart from the possible influence of the gut microbiota on the bioavailability of oral drugs mentioned above, the influence on some special drugs such as digoxin, berberine and levodopa is representative and may give us some inspiring and useful advice on clinical treatment.

5.1. *The influence on the inactivation of digoxin*

Digoxin is a widely used cardiovascular drug treating patients for heart failure and atrial fibrillation[147]. However, pronounced interindividual variability in the bioavailability of digoxin has been observed for a long period. After digoxin is administered orally, about 10% of patients convert the drug substantially to cardioinactive, reduced metabolites (digoxin reduction products, or DRPs), such as dihydrodigoxin in which the single double bond in the lactone ring of digoxin is reduced[43,44]. Since DRPs poorly bind to the cardiac receptor site (membrane-associated Na^+,K^+-dependent adenosinetriphosphatase), only a minority of them are concentrated by cardiac tissue and they can be excreted rapidly, exerting much less cardiac therapeutic efficacy compared with digoxin[44]. This phenomenon draws a lot of researchers' attention to investigate the mechanisms of digoxin inactivation, especially when the therapeutic range of digoxin is exceedingly narrow (0.5−2 ng/mL)[79]. In 1981, the gut microbiota was initially found to play a part in the inactivation of digoxin[43]. In 1983, *Eubacterium lentum* (now known as *Eggerthella lenta*) which was responsible for the reduction of digoxin was isolated and identified[44]. However, it was found that not all strains of *E. lenta* could inactivate digoxin, because high concentrations of these organisms were also found in the stools of individuals who did not excrete DRPs[44]. In 2013, specific *E. lenta* strains inducing the inactivation of digoxin were discovered. By the application of transcriptional profiling, comparative genomics and culture-based arrays, these specific strains were identified as possessing a two-gene cardiac glycoside reductase (*cgr*) operon which could be up-regulated by digoxin and act as potential microbial biomarkers for digoxin inactivation[45]. According to sequence homology (PSI-BLAST) and secondary structure predictions (HHPred), the *cgr* operon is predicted to encode two kinds of proteins. One is CGR1 which is homologous to the NapC/NirT family of cytochrome c reductases, the other one is CGR2 related to fumarate reductases[47]. CGR1 shuttles electrons from quinones to associated terminal electron reductase partner. Forming a complex with CGR1, CGR2 may act as that terminal electron reductase partner receiving electrons from CGR1 at the active site FAD redox cofactor. In CGR2, there is a digoxin binding pocket which primarily contains the negatively charged polar amino acids and a few non-polar hydrophobic residues[46]. Digoxin can be reduced to DRPs by CGR1/CGR2 complex at the active site of CGR2[47]. Although arginine is found to be required for the growth of *E. lentad*[148], it can inhibit the conversion of digoxin to inactive forms[44,45]. Therefore, having a high-protein (high-arginine) diet may lead to better bioavailability of digoxin and is recommended to patients taking digoxin, which indicates that diet may also have an influence on the microbial drug metabolism[55].

5.2. *The influence on the absorption of berberine*

The gut microbiota plays a crucial part in the absorption process of berberine (BBR). BBR is a medicinal alkaloid isolated from *Coptis chinensis*. Due to its antimicrobial and antidiarrheal activity, it has been used orally as an over-the-counter (OTC) drug in China to treat patients for diarrhea with good safety for decades[48,149]. Apart from diarrhea, BBR has been proved to be able to treat patients suffering from hyperlipidemia and T2D for it can reduce blood lipids and glucose in patients *via* a multiple-target mechanism[150−153]. Although the efficacy of BBR is desirable, its bioavailability is less than 1% possibly due to its poor water solubility, poor intestinal permeation through the paracellular pathway and rapid biotransformation[154]. Loads of research has been conducted to explore the exact mechanism of BBR absorption and try to explain why it can possess excellent efficacy while its bioavailability is so low. Feng et al.[48] demonstrated that nitroreductases (NRs) of the gut microbiota were key to BBR absorption. After orally administered, BBR was found to be reduced to dihydroberberine (dhBBR), an absorbable form whose intestinal absorption rate was 5-fold higher compared with BBR in animals, by gut microbial NRs. dhBBR was only a transient form which can be reverted to BBR quickly in intestine tissues by the oxidization which was proved to be a non-enzymatic reaction, and then BBR entered the blood. The decreased BBR plasma concentration and reduced BBR therapeutic efficacy in hyperglycemia and hyperlipidemia were reported in mice treated with antibiotics before and during BBR treatment. This drug−drug interaction needs to be noticed when antibiotics are prescribed to patients who are receiving BBR treatment, because the clinical therapeutic efficacy may be impaired due to the antibiotic-mediated alterations in the gut microbiota. Interestingly, as NRs are assumed to facilitate the general detoxification of bacteria[155], the author suggested that the conversion of BBR to dhBBR might be a self-protection mechanism of the gut microbiota[48]. And this may be the outcome of the long-term symbiosis between the gut microbiota and the host. They also investigated the pharmacokinetics of BBR in beagle dogs and found that apart from being reduced by gut bacteria, BBR could also facilitate the production of butyrate by intestinal microbiota, which was associated with the anti-hyperlipidemia effect of BBR[156]. In addition, the researchers observed an intriguing effect about BBR in hamsters fed with high fat diet (HFD). An increase in blood lipids and the NR activity

was found in HFD-fed hamsters. Both the increase of NR-producing gut microbiota and the enhancement of their NR activity contributed to the elevation of fecal NRs by HFD-fed hamsters. Besides, after being administered orally, the bioavailability of BBR was higher in HFD-fed hamsters than that in those with normal diet. And the decrease of blood lipids by BBR was only found in HFD-fed hamsters. The results of clinical trials they conducted were consistent with this effect. The higher fecal NR activity and a higher level of BBR were observed in hyperlipidemic patients compared with healthy individuals. Since a positive relationship between the BBR blood level and the fecal NR activity was observed, the fecal NR activity has the potential to serve as a biomarker to predict the therapeutic efficacy of BBR and instruct the personalized treatment of hyperlipidemia using BBR in clinical practice[157].

5.3. *The influence of H. pylori infection on the absorption of levodopa*

H. pylori (HP) infection can impair the absorption of levodopa which is a classic drug used to treat patients for Parkinson's disease (PD). In a placebo-controlled crossover study conducted in 2001, the mean AUC of levodopa increased by 21% after a seven-day HP eradication therapy with omeprazole, amoxicillin and clarithromycin. The clinical symptoms were also improved after the use of antibiotics[51]. In 2006, this research group conducted a randomized controlled trial with 34 PD patients who were randomized divided into two groups. One group received a 7-day course of omeprazole, amoxicillin and clarithromycin, while the other group was treated with an antioxidant (allopurinol) for 15 days. A 54% increase in levodopa absorption in the PD eradication group was observed coupled with a great improvement in clinical disability[28]. Another clinical trial also showed that levodopa onset time, ON duration, motor severity and quality of life parameters were improved after the eradication of HP[49]. These trials indicate that the eradication of HP can improve the levodopa absorption, which may achieve better treatment for PD. The exact mechanism(s) of this effect has not been elucidated yet, but there are some possible hypotheses based on the HP infection-induced alterations of the gut intestinal tract's environment. HP may change gastric motility, disrupt the duodenal mucosa[158], and produce reactive oxygen species[159], which might result in a decrease of levodopa bioavailability. The solubility of levodopa is pH-sensitive and the gastric acidity can be changed by the HP infection, which might potentially lead to the impairment of levodopa absorption. Besides, the HP infection can increase the level of gastric istidina decaboxylase which shares approximately 50% of amino acid sequence with L-dopa decarboxylase. This may facilitate the gastric biotransformation of levodopa to dopamine which could not be absorbed[51]. In addition, an *in vitro* experiment demonstrated a direct interaction between levodopa and some surface adhesins of HP. Due to this interaction, the free levodopa concentration in bacterial suspension decreased significantly, which may provide a possible explanation for the reduction of levodopa absorption. However, the kinds of relevant adhesins were not identified in the experiment[50]. As it has been confirmed in some clinical trials that HP infection is more prevalent in patients suffering from Parkinson's disease[160,161], the eradication of HP may be necessary to achieve a better outcome of PD treatment. More relevant research investigating the basic mechanism(s) is still in need to prove this effect.

6. The influence of drugs on the gut microbiota

Apart from antibiotics, some kinds of drugs can also alter the composition of the gut microbiota, and then may influence the bioavailability of oral drugs. Since these drugs, especially drugs for chronic diseases, have a great chance of being administered with other oral drugs, we need to pay attention to the potential drug−drug interaction which they may induce *via* their effect on the gut microbiota. Some clinical trials on how the drugs influence the human gut microbiota are listed in Table 3.

Proton pump inhibitors, a kind of antiulcer/antireflux drugs, can change the composition of the gut microbiota by altering pH of the GI tract and delaying gastric emptying rate[177]. It was found that there was a difference in the relative abundance of 20% of the bacterial taxa, whereof 18 bacterial families between people using PPIs and non-users[178]. The PPIs use leads to relative excess of *Streptococcus*, regardless of *H. pylori* status, which may result in dyspeptic symptoms in PPIs users[179]. Besides, many psychoactive drugs have exhibited antibacterial activity *in vitro via* changing bacterial morphology, inhibiting cell wall synthesis or the antiplasmid activity[158]. However, the drug dose used in some *in vitro* experiments is above the dose used in clinical treatment, so whether the clinical doses of these drugs can still have the antibacterial activity needs to be investigated[158]. Lukić et al.[180] demonstrated that abundances of *Ruminococcus*, *Adlercreutzia*, and an unclassified Alphaproteobacteria were reduced by antidepressants. Interestingly, they found that *Ruminococcus flavefaciens* could abolish the effects of antidepressants on depressive-like behavior, indicating that reducing *R. flavefaciens* might result in better therapeutic efficacy of antidepressants.

Some antidiabetic drugs can also influence the composition of the gut microbiota. It has been found that metformin, an antidiabetic drug belonging to biguanide class, can increase the abundance of *Akkermansia muciniphila* which is reduced during diabetes and obesity[181] and several short-chain fatty acids (SCFA)-producing bacteria[182,183]. *A. muciniphila* acts a pivotal part in maintaining the mucin layer integrity, by which proinflammatory lipopolysaccharides translocation is reduced and fat storage, adipose tissue metabolism, and glucose homeostasis are controlled[182,183]. Besides, Plovier et al.[181] isolated a purified membrane protein, Amuc_1100, from *A. muciniphila*, and found that it could improve metabolism in mice with obesity and diabetes. It has been reported that intestinal gluconeogenesis can be triggered by the increase in SCFAs produced in the colon,

Table 3 Clinical trials on the drug-induced alterations in the gut microbiota.

Therapeutic class	Drugs investigated	Clinical study
Antiulcer/antireflux	Various proton pump inhibitors	162−165
Antidiabetic	Metformin	166−168
	Acarbose	169
Antipsychotic	Atypical antipsychotics	170,171
	Risperidone	172,173
	SSRI antidepressants	174
	Olanzapine	175
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Various NSAIDs	176

especially butyrate and propionate, inducing metabolic benefits in energy homeostasis such as a decrease in adiposity and body weight, and a better glucose control, including reduced hepatic glucose production[184]. All these findings prove that the alteration in the gut microbiota caused by metformin may inversely improve the antidiabetic effects *via* an increase in gastrointestinal mucins and SCFAs production. Acarbose can also have an influence on the gut microbiota composition and it increases the abundance of SCFA-producing bacteria including *Faecalibacterium*, *Prevotella* and *Lactobacillus*[182]. The possible mechanism of the alteration may be that the complex carbohydrates' digestion can be delayed by acarbose as acarbose can inhibit pancreatic α-amylase and some α-glucosides. Then these carbohydrates can be fermented by the gut microbiota, which may change the composition[182]. A cross talk between acarbose and the gut microbiota may be existed. There may be the relevance of acarbose's effect on body weight loss to the alteration in the gut microbiota composition and it has been reported that the increase of SCFAs may result in longer lifespan of mice treated with acarbose[182].

The gastrointestinal pH can be indirectly changed because of the increased production of SCFAs induced by drugs[158], which may potentially influence the bioavailability of some oral drugs. Since the alterations in the gut microbiota induced by metformin and acarbose both lead to a higher level of SCFAs and the increased abundance of *A. muciniphila* induced by metformin leads to the change in the mucin layer, some oral drugs' bioavailability may be affected due to the change in these gastrointestinal physiological properties. Besides, the expression of some gut microbial genes related to drug metabolism can be influenced by some drugs, such as sulfasalazine and nizatidine. The expression of thioredoxins and nitrate reductases can be induced by sulfasalazine while the expression of drug enzymes cleaving nitrogen bonds can be up-regulated by nizatidine[158]. This kind of drug−drug interaction *via* the alteration in the gut microbiota should be noted in the clinical treatment. When drugs whose bioavailability is subject to the gut microbiota are prescribed with drugs which may lead to the dysbiosis of the gut flora, probiotics can be co-administered to facilitate the restoration of the gut microbiota. And for those whose pharmacokinetics may be influenced by the drug-induced change in the gut microbiota, the clinical dose should be rationally adjusted. However, the relationship between oral drugs' bioavailability and the drug-induced alteration in the gut microbiota has not been clear yet. More animal studies and intervention studies are still in need to offer directions to the co-administration of oral drugs in the clinical treatment.

7. The influence of the gut microbiota on the drug delivery system design

The influence of the gut microbiota on the bioavailability of oral drugs provides some useful advice and strategies for the drug delivery system design, especially for the design of the colon-targeted drug delivery system. For the colon-targeted drug delivery, it is important to avoid the drug being absorbed in the upper intestinal tract while the drug needs to be released in time at the colon site. Since the applications of pH-dependent and osmotic controlled release systems have many limitations like irregular pH changes and variability in GI transit time, the microbiota-activated delivery system taking advantage of the bacterial enzyme activity seems to be the most promising colon-targeted delivery system[16,185]. By investigating the recent research, we can briefly sort microbiota-activated delivery systems into three categories: the prodrug, the azo polymeric drug delivery system and the polysaccharide-based drug delivery system.

7.1. *The prodrug*

Apart from the azo prodrugs mentioned above, different types of molecules with various chemical structures are used by researchers as carriers to deliver the active moiety direct to the colon. Kim et al.[186] used amisulpride (ASP), an anti-psychotic agent, as a carrier to deliver 5-aminosalicylic acid (5-ASA) to the colon as a potential prodrug, ASP-azo-ASA, to treat patients with IBD. The research showed that ASP-azo-ASA had the better ability to decrease the levels of inflammatory mediators in the inflamed distal colon than SSZ and the ASP systemic absorption was limited. Nalinbenjapun et al.[187] conjugated 5-ASA to chitosan through a 4-aminobenzoyl spacer. However, this new kind of azo prodrug only released 25% of the 5-ASA load in simulated colonic fluid with rat colon content in 24 h while SSZ released around 70% of the drug load during the same time period. The researchers suggested that it might be a good approach to elevate the release of 5-ASA by formulating this chitosan-5-ASA conjugate as nanoparticles in order to enhance the chance of the azo bond exposure to the bacterial azoreductases. This example also gives us an innovative idea that we might be able to increase the efficacy of prodrugs by formulating them in the range of nanosize. In addition to azo prodrugs, there are other prodrug systems like amino acid conjugates, glycoside conjugates, glucuronide conjugates, cyclodextrin conjugates and acetic acid conjugates[16].

It has been reported that some gut microbiota such as *Clostridium*, *Salmonella*, *Bifidobacterium*, *Listeria* and *E. coli* can accumulate and even proliferate in the tumor microenvironment (TME), making it possible for the bacteria-directed enzyme prodrug therapy (BDEPT). In BDEPT, the genetically engineered bacteria expressing specific prodrug-activating enzymes are administered to the patients and they can accumulate and secrete prodrug converting enzymes within TME. When the level of enzymes is optimum, the prodrug is given to patients and it can be converted to the active form specifically within TME[188]. Afkhami-Poostchi et al.[188] demonstrated a novel BDEPT approach using a genetically engineered *E. coli* DH5α-lux/βG accompanied by glycyrrhizic acid. Glycyrrhizic acid could be converted to glycyrrhetinic acid which possessed a better anti-cancer effect by β-glucuronidases expressed by *E. coli* DH5α-lux/βG in TME. They evaluated this approach in a mouse model with colon carcinoma, and it showed a significant ability to suppress the growth of tumor with less toxicity than the treatment with bacteria or prodrug alone, offering an effective strategy for cancer-targeted therapy.

However, prodrugs have some limitations on the drug delivery system design. Only a few drugs possess the appropriate structure to construct prodrugs and the toxicity of them should be investigated before the clinical use.

7.2. *The azo polymeric drug delivery system*

Taking advantage of the bacterial azoreductase activity, many polymer materials possessing the azo bond are used to design colon-specific drug delivery systems. Hou et al.[189] synthesized a

Table 4 The polysaccharide-based drug delivery systems.

Polysaccharide	Delivery system	Drug/payload molecule	Therapeutic application	Feature	Ref.
Chitosan	Chitosan-capped enzyme-responsive hollow mesoporous silica nanoplatforms	Doxorubicin	Not mentioned in the literature	Bacterial enzyme sensitive	192
Chitosan	Eudragit S-100 and chitosan-based nanoparticles	Paclitaxel	Colorectal cancer	Sustained release, pH responsive, bacterial enzyme sensitive and cancer-targeted	193
Amphipathic chitosan derivative	Amphiphilic chitosan derivatives and cell-penetrating peptides modified nanoparticles	Insulin	Lowering the blood glucose	The bacterial enzyme sensitive delivery and the use of cell-penetrating peptides	194
Chondroitin sulfate	Tablets coated by Surelease® and chondroitin sulfate	Dapsone	Not mentioned in the literature	Time-dependent and bacterial enzyme sensitive	195
Dextran	The doxorubicin and superparamagnetic iron oxide nanoparticles-loaded solid lipid nanoparticle coated with folate and dextran	Doxorubicin and superparamagnetic iron oxide nanoparticles	Colon cancer	The microbial enzyme sensitive and tumor-targeted delivery system used for chemo/magnetothermal combination therapy	196
Guar gum	The guar gum modified upconversion nanocomposite	5-Fluorouracil	Colorectal cancer	Bacterial enzyme sensitive and NIR triggered	197
Guar gum	Transformable capsules containing indomethacin immediate-release pellets	Indomethacin	Colon cancer	Bacterial enzyme sensitive	198
Guar gum	Microspheres	Mesalamine and synbiotics	Ulcerative colitis	Bacterial enzyme sensitive	199
Guar gum	5-Flurouracil-containing mesoporous silica nanoparticles with guar gum capping	5-Flurouracil	Colon cancer	Bacterial enzyme sensitive	200
Pectin	The pectin/modified nano-carbon sphere nanocomposite gel films	5-Fluorouracil	Colon cancer	Bacterial enzyme sensitive	201
Pectin	Hybrid pectin/CMC—Na microspheres (MS) cross-linked with Zn^{2+} and Al^{3+}	Progesterone	Gynecological disorders	pH responsive, bacterial enzyme sensitive, and mucoadhesive	202
Pectin	Pectin–Zinc acetate beads coated with Eudragit S100	Pterostilbene	Colorectal cancer	pH responsive and bacterial enzyme sensitive	203
Pectin	Pectin/poly (ethylene glycol) (PEG) hydrogel containing *in situ* mineralized calcium carbonate microparticles	Bovine serum albumin	Not mentioned in the literature	pH responsive and bacterial enzyme sensitive	204
Starch	Phloral™ coated tablets	5-Aminosalicylic acid	Not mentioned in the literature	pH responsive and bacterial enzyme sensitive	205
Starch	OPTICORE™ coating tablets	5-Aminosalicylic acid	Not mentioned in the literature	pH responsive and bacterial enzyme sensitive	206
Starch	Injection molded capsules	Acetaminophen	Not mentioned in the literature	Time-controlled and bacterial enzyme sensitive	207
Chitosan and alginate	Thiolated chitosan/alginate composite microparticulates coated by Eudragit S-100	5-Aminosalicylic acid and curcumin	Colitis	pH responsive, bacterial enzyme sensitive, and mucoadhesive	208
Chitosan and sodium alginate	The sodium alginate-coated electrospun fiber mat containing quercetin-loaded chitosan nanoparticles and prebiotics	Quercetin and prebiotics	Colon cancer	Bacterial enzyme sensitive	131
Chitosan succinate and sodium alginate	Capecitabine encapsulated chitosan succinate-sodium alginate macromolecular complex beads	Capecitabine	Colon cancer	pH responsive, bacterial enzyme sensitive, and mucoadhesive	209
Chitosan and alginate	Microcapsules	Interleukin-1 receptor	Inflammatory bowel disease	pH responsive and bacterial enzyme	210

(*continued on next page*)

Table 4 (*continued*)

Polysaccharide	Delivery system	Drug/payload molecule	Therapeutic application	Feature	Ref.
		antagonist		sensitive	
Chitosan and pectin	Modified citrus pectinate–chitosan nanoparticles	Cetuximab and curcumin	Colon cancer	Bacterial enzyme sensitive, mucoadhesive and tumor-targeted	211
Chitosan and pectin	Nanoparticles	Berberine	Obesity and associated metabolic disorders	pH responsive and bacterial enzyme sensitive	212
Sodium alginate and Portulaca polysaccharide	Polymeric beads encapsulating 5-fluorouracil	5-Fluorouracil	Colorectal cancer	pH responsive and bacterial enzyme sensitive	213
Guar gum and pectin	Tablets coated with guar gum and Eudragit S100	Modified apple polysaccharide and mesalamine	Ulcerative colitis	Bacterial enzyme sensitive	214
Hyaluronic acid and chitosan	Hyaluronic acid-coupled chitosan nanoparticles bearing oxaliplatin encapsulated in Eudragit S100-coated pellets	Oxaliplatin	Colon cancer	Bacterial enzyme sensitive	215
A novel polysaccharide from *Trigonella foenum-graecum*	Compression-coated tablets	Metronidazole	Localized treatment of amoebiasis	pH responsive and bacterial enzyme sensitive	216

NIR, near infrared.

pH-sensitive and enzyme-sensitive nanocomposite hydrogel using graphene oxide (GO) containing azoaromatic crosslinks as well as poly (vinyl alcohol) (PVA) (GO−N═N−GO/PVA composite hydrogels) to deliver curcumin as a treatment for colon cancer. It showed that this kind of hydrogel could protect curcumin from being released before reaching the colon and it exhibited a better colon-targeting capacity and longer residence time in the colon. Ma et al.[190] synthesized an enzyme-sensitive and pH-sensitive controlled release hydrogel by using an acryloyl chloride modified olsalazine as an azo crosslinker copolymerized with hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA) to deliver 5-flurouracil. This azo hydrogel showed a pH-responsive swelling pattern and the local release of 5-flurouracil in the colon, indicating that it might be a potential effective drug delivery system targeting colon. With the cross-development of polymer materials science, medical and biological science, a number of innovative azo polymers will be synthesized and be applied to the design of colon-specific drug delivery system.

7.3. *The polysaccharide-based drug delivery system*

For the polysaccharides can be hydrolyzed into monosaccharides by *Bacteroids* and *Bifidobacterium*, they are always used as a kind of safe, non-toxic material for colon-targeted drug delivery systems[16,56]. There are many types of polysaccharides which can be used, like chitosan, pectin, inulin, guar gum, dextrans and alginate[191] and the recent polysaccharide-based drug delivery systems are listed in Table 4.

It is an effective strategy to modify drug delivery systems with polysaccharides on the surface to protect some peptides or ligands from damaging in the upper gastrointestinal tract. Guo et al.[194] designed a colon-targeted nanoparticle co-modified with amphipathic chitosan derivative (ACS) and cell-penetrating peptide (CPP) to improve the bioavailability of insulin. In the upper gastrointestinal tract, ACSs modified on the surface of the nanoparticles prevented CPPs from degradation. After reaching the colon, ACSs were degraded by the gut microbial enzymes and CPPs were exposed to facilitate the colon epithelium penetration, resulting in better oral absorption of insulin. Polysaccharides can also prevent ligands from being exposed in the stomach and small intestine to realize the colon-specific and cancer-targeted drug delivery. Shen et al.[196] reported a solid lipid nanoparticle (SLN) loading doxorubicin and superparamagnetic iron oxide nanoparticles to be used as the chemo/magnetothermal combination therapy. The SLNs were first modified with folate (FA) and then were coated with dextran on the surfaces to prevent FA from being transported by the FA transporter in small intestine and enhance the SLNs residence in colon. In the colon, dextran shells were damaged by bacterial enzymes and FA was exposed to achieve colon cancer-targeted delivery.

Polysaccharides can also be used to design dual-stimuli responsive drug delivery systems. A novel, colon-targeted delivery system (CODES™, Fig. 6) is a technology which combines the features of polysaccharides that can be degraded to organic acids in the colon and pH-sensitive characteristics of coating polymer, which can ensure the release of drugs at specific sites[217]. Phloral™[205] and OPTICORE™[206] are two similar technologies which are also both bacterial enzyme sensitive and pH sensitive. In order to prevent enzymatic degradation, stomach irritation caused by BBR and complex drug−drug interaction, Guo et al.[212] designed a microbiota-targeting and colon-specific BBR delivery system using chitosan (CS) and pectin (PT). This novel delivery

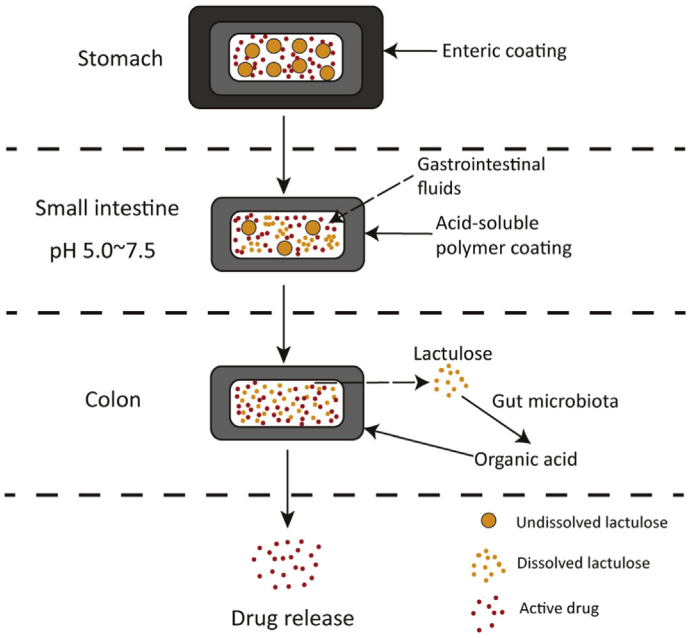

Figure 6 Schematic illustration of the principle of colon-targeted delivery system (CODES™) technology. There are three main parts of the system. Lactulose and the active drug are contained in the inner core, which is coated with a layer of acid soluble material, and then further coated with another layer of enteric material. The enteric coating protects the tablet while it is in the stomach and then dissolves when the tablet reaches the small intestine. Because of the acid-soluble polymer coating, the drug could not release from the tablet, while gastrointestinal fluids can penetrate through the coating layer to dissolve the lactulose. After reaching the colon, the gut microbiota can degrade lactulose diffusing through the coating to produce organic acids. This process results in a lower pH of the local environment which leads to the dissolution of the acid-soluble coating and the release of the drug.

system, BBR-CS/PT-NP, is pH/gut microbiota dual stimuli-responsive. Due to the strong electrostatic attractions between chitosan and pectin, BBR could not be released from nanoparticles in the stomach and small intestine. Because of the alteration in the pH value after BBR-CS/PT-NPs reaching the colon, the electrostatic interaction becomes weaker due to the deprotonation of chitosan. Furthermore, both chitosan and pectin can be degraded by the gut microbiota in the colon, so there is a burst release of BBR in the colon site. BBR-CS/PT-NPs might have the ability to treat patients for obesity and associated metabolic disorders by modulating the gut microbiota. The enhanced interaction between BBR and the gut microbiota might contribute to the better therapeutic efficacy of BBR-CS/PT-NPs compared with BBR solution. This research also indicates that the gut microbiota may be a novel target to be aimed at in the design of drug delivery systems for the treatment of some metabolic diseases.

The composition of the gut microbiota is dynamic and it can be affected by diet, disease or drug therapy. The disease like IBD or the drugs which are used to treat some gastrointestinal diseases, such as 5-ASA[218] and mesalamine[219], may alter the gut microbial flora and then affect the degradation rates of the polysaccharide-based drug delivery system. The co-administration of the synbiotics consisting of probiotics and prebiotics may be a good solution to improve this situation for it can help refurbishing the gut microbiota. Kaur et al.[199] formulated mesalamine as microspheres with guar gum and xanthan gum combined with probiotics including *L. acidophilus*, *L. rhamnosus*, *Bifidobacterium longumand* and *Saccharomyces boulardi* as the treatment for ulcerative colitis. The polysaccharides acted as prebiotics and facilitated toping up the normal microbiota of colon with probiotics together, achieving more efficient colon-targeted drug delivery. This instance also inspires us that we need to take the possible influence of diseases and drugs delivered on the gut microbiota at the beginning of the drug delivery system design.

Although polysaccharide-based drug delivery system has the merits like achieving site-specific delivery of drugs and good biocompatibility, it still has some limitations like the high water solubility and the poor film forming property which can be improved respectively by chemical derivatization/crosslinking and forming mixed films using synthetic film forming polymers[185,220].

8. Conclusions and future perspectives

The gut microbiota can influence the bioavailability of oral drugs mainly *via* the alteration in the metabolism catalyzed by gut microbial enzymes, the regulation of the host's genes expression, substrate competition and affecting the intestinal properties. Apart from indigenous gut microbiota, the infection of *H. pylori* and the supplement of probiotics can also change the pharmacokinetics of oral drugs. Besides, drug−drug interaction may be induced by the alteration in the gut microbiota, which needs to be noted in the clinical treatment. The possible relationships between the gut microbiota and the bioavailability of oral drugs are presented in Fig. 7. However, there has not been enough literature on this topic up to now. Although there is some research providing evidence of the relationship between the gut microbiota and the bioavailability of oral drugs, most of the conclusions are still in a primary stage and the exact mechanisms for many effects remain unknown. Besides, many results are strain-specific or drug-specific, so it is

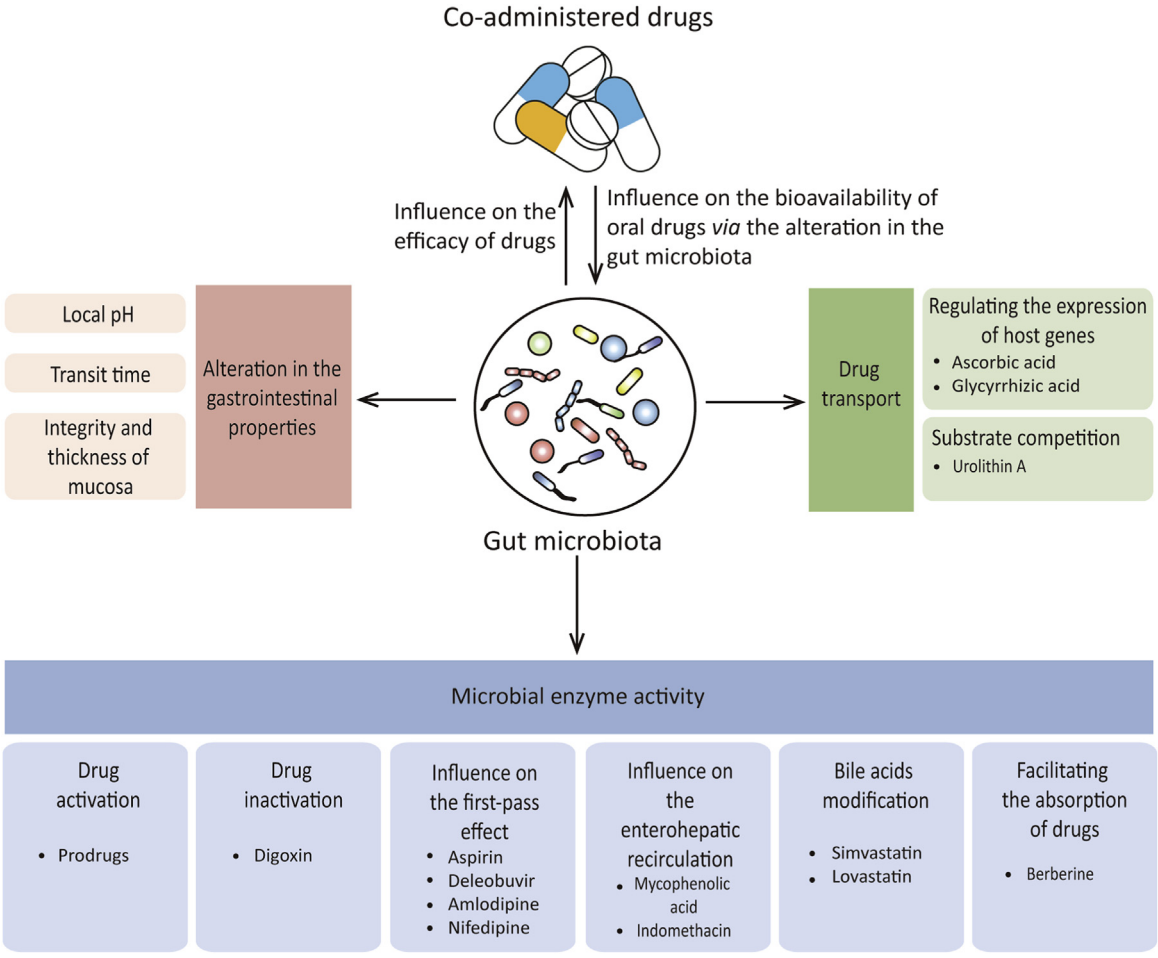

Figure 7 Possible relationships between the gut microbiota and oral drugs' bioavailability.

hard to extrapolate general conclusions from those results. It is also noticeable that the majority of the experiments are either *in vitro* or carried out in animals. As there are some differences in the composition or the enzyme activity of the gut microbiota and some physiological characteristics between animals and humans, it is still hard to predict the actual effects in human bodies according to the existing research. As bioavailability is of great importance to the therapeutic efficacy of oral drugs, especially to those whose main absorption sites are in the lower gastrointestinal tract, it is necessary to explore the mechanisms that can explain those effects caused by the gut microbiota with the help of advanced technologies, such as high-throughput DNA sequencing technology, hybridization methods and the use of flow cytometry with tandem mass spectrometry[221]. *In vivo* experiments and clinical trials are also in need to prove those assumptions and preliminary conclusions. In addition, since excipients are essential in the design of oral drug formulations, more work should be done to investigate the possible interaction between them and the gut microbiota. Understanding the influence of the gut microbiota on the bioavailability of oral drugs provides new ideas for the colon-targeted drug delivery system design and the adjustment of oral drug formulations with better efficacy. It can also give practical advice on the co-administration of antibiotics and other chronic drugs with oral drugs whose bioavailability is susceptible to the gut microbiota in order to prevent the undesired drug−drug interaction. Besides, the clinical dose of some drugs may need to be adjusted in some specific conditions, such as plateau hypoxia or co-administered with some drugs. Because explorations of this topic may provide predictive biomarkers and make it possible to evaluate the oral drugs' bioavailability by modulating the gut microbiota, continuous research will promote the development of personalized medicine to achieve the more precise and better clinical therapeutic efficacy.

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Author contributions

Zhonggao Gao and Xintong Zhang chose the topic and designed the outline for this review. Xintong Zhang wrote the manuscript. Xintong Zhang and Ying Han drew the figures and designed the tables. Ying Han, Wei Huang and Mingji Jin revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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