Mechanisms of gastrointestinal microflora on drug metabolism in clinical practice

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Abstract

Considered as an essential "metabolic organ", intestinal microbiota plays a key role in human health and the predisposition to diseases. It is an aggregate genome of trillions of microorganisms residing in the human gastrointestinal tract. Since the 20th century, researches have showed that intestinal microbiome possesses a variety of metabolic activities that are able to modulate the fate of more than 30 approved drugs and immune checkpoint inhibitors. These drugs are transformed to bioactive, inactive, or toxic metabolites by microbial direct action or host-microbial co-metabolism. These metabolites are responsible for therapeutic effects exerted by these drugs or side effects induced by these drugs, even for death. In view of the significant effect on the drugs metabolism by the gut microbiota, it is pivotal for personalized medicine to explore additional drugs affected by gut microbiota and their involved strains for further making mechanism clear through suitable animal models. This review mainly focus on specific mechanisms involved, with reference to the current literature about drugs metabolism by related bacteria or its enzymes available.

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1. Introduction

Considered as an essential “metabolic organ”, intestinal microbiome contains more than 1000 bacterial species whose total number is as high as 100 trillion, and has approximately 150 times the genetic capacity of the human genome (Ley et al., 2006; Qin et al., 2010; Tilg and Kaser, 2011; Lakshminarayanan et al., 2014). A large section of healthy intestinal microbiome comprises bacteria, with the phyla Firmicutes and Bacteroidetes being the most dominant, and a smaller section is composed of archea, protozoa, viruses and fungi (Eckburg et al., 2005; Ley et al. 2005; Sommer and Bäckhed, 2013). These microorganisms colonize the host body from birth, and their diversity then increases considerably at the strain level, to the point where adults have relatively stable gut microbiota (Arumugam et al., 2011). However, each individual has a specific microbial taxa (Costello et al., 2009; Wu et al., 2011), and even twins have <50% of the same bacterial species (Turnbaugh et al., 2010), which is influenced by lots of factors including host genotype (Benson et al., 2010), antibiotic usage (Jernberg et al., 2010; Dollive et al., 2013), concomitant diseases (Zhao, 2013), and the maternal environment (Behnsen et al., 2013). The unique community is indispensable for maintaining human health (Clemente et al., 2012). It can ferment proteins and carbohydrates that are not digested by host into absorbable energy and short chain fatty acids (SCFA) (Ramakrishna, 2013), maintain mucosal structure to shape the immune response (Artis, 2008), synthetize vitamins (B and K) (Ferreira et al., 2011), and metabolize many endogenous substances including cholesterol, bile acids, tryptophan, neurotransmitters (Kinross et al., 2011). These microorganisms and their metabolites are also considered important in metabolizing orally administered exogenous compounds, especially drugs (Jeong et al., 2013)). Therefore, inter-individual or intra-individual microbial diversity or dysbacteria is of potential clinical significance.

It is well known that the liver is a main organ responsible for pharmaceutical metabolism and biotransformation. However, the metabolism of drug may begin at the intestinal level earlier than that at liver. Great majority of intestinal microbes reside in colon where the quantity of microorganisms is as high as 10^{13}. Microbial distribution is characterized by a decrease in the quantity and species of microorganisms from the colon to the stomach (Sender et al., 2016). Oral delivery of medicines, especially those that are not completely absorbed from the small intestine or candidates for enterohepatic circulation, are in inevitable contact with microbiota of the distal gut (Moore, 2008). And the metabolic response performed by gut microbiota is completely different from that by the liver. Specifically, the microbiota mainly produces non-polar low-molecular weight metabolites through hydrolytic and reductive metabolism, while the liver primarily generates polar and high-molecular weight byproducts through oxidative and conjugative metabolism (Joh and Kim, 2010; Kim, 2015). Thus, the intestinal microbiota affects drug absorption, and changes pharmacological effects of drugs. The effect of gut microbiota on host-targeted drugs efficacy has long been recognised in the late 1930s (Mani et al., 2014), when the sulfa-based antibiotics are identified as substrates for microbial transformation. It is attributed to microbial estimated 3.3 million genes, particularly genes encoding heterogeneous biodegradation and metabolic pathways (Qin et al., 2010). In recent years, a large number of studies on drug metabolism mechanisms have proved that the intestinal microbiota possesses a variety of metabolic activities that are able to modulate the fate of more than 30 approved drugs and their bioavailability (Okuda et al., 1998; Clayton et al., 2009; Haiser et al., 2014). These medicines are transformed to bioactive, inactive, or toxic metabolite(s) (Jeong et al., 2013; Yoo et al., 2014). For instance, the enzymes from colonic bacteria catalyze the reduction of sulfasalazine into 5-aminosalicylic acid, which induces anti-inflammatory effects (Klotz, 1985; Hayllar and Bjarnason, 1991). And gut microbiota metabolizes levamisole into three activity-reduced metabolites (Shu et al., 1991). Other representative examples of pharmaceuticals metabolized by the gut microbiota include diclofenac, omeprazole, digoxin, irinotecan, glycyrrhizin and so on.

In addition, the metabolic association between the host and gut microbiota changes the pharmacokinetics and pharmacodynamics of drugs (Haiser and Turnbaugh, 2012; Carmody and Turnbaugh, 2014), such as acetaminophen. In view of the significant effects on drug metabolism by gut microbiota, this review mainly focus on specific mechanisms involved, with reference to current literature about drugs metabolism whose related bacteria or enzymes are available.

2. Diverse mechanisms of intestinal microflora affecting drug metabolism

In fact, all functional activities performed by humans beings, including drug metabolism, are determined by two sets of genomes – the genetically inherited human genome and the environmentally acquired microbiome (Lederberg, 2000). Among all environmentally-acquired microorganisms, intestinal microorganisms are most abundant. Accumulating evidences reveal that changes in the gut microbiome are linked to many diseases, including diabetes, obesity (Forslund et al., 2017), CDI (Ferreira et al., 2014), cardiovascular disease (Singh et al., 2016) and inflammatory skin disease (2018). And its diversification contributes to the interindividual differences of results with drugs therapy. Together, a wealth of information is emerging about the roles of the gut microbiota on pharmacokinetics and therapeutic outcomes, particularly on drugs metabolized by particular bacteria or known bacterial enzymes. There are also some medicines that are confirmed to be associated with the intestinal microbiota by animal models, but their associated microbiota and bacterial metabolites are unknown. Due to the differences in the quantity and diversity of intestinal microbiota in animal models and human, and various factors affecting pharmacokinetics in vivo, the specific correlation between these medicines and human intestinal microbiota needs further investigation. When we focus on drugs that are clearly related to the intestinal microbiota, we find out the bacteria participate in the biotransformations of administered drugs, even dietary compounds and environmental toxins, through a number of diverse direct or indirect mechanisms (Carmody and Turnbaugh, 2014; Klaassen and Cui, 2015). Several articles have reviewed the effects of gut microbiota on efficacy and toxicity of dietary components and environmental toxins (Spanogiannopoulos et al., 2016; Wilson and Nicholson, 2017). The list of drugs and phytochemicals that are metabolized by the specific gut microbiota or microbial enzyme through known mechanism is presented in Table 1.
Drugs and phytochemicals that are metabolized by the specific gut microbiota or microbial enzyme.

| Drug                        | Clinical application | Bacterial Species or enzymes involved | Mechanism                                                                 | Ref.                                                                 |
|-----------------------------|----------------------|---------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------|
| Prontosil                   | Antibiotics          | Azoreductases                         | Enzymatic cleavage of azo-bond to sulfanilamide                          | Hattori et al. (1980, 1983), Haylillar and Bjarnason (1991) and He et al. (2017) |
| Neoprontosil                |                      |                                       |                                                                           | Hattori et al. (1980, 1983) and Haylillar and Bjarnason (1991)          |
| Sulfasalazine               | Anti-inflammatory    |                                       | Enzymatic cleavage of azo-bond to sulfanilamide 5-aminosalicyclic acid   | He et al. (2017)                                                       |
| Balsalazide Olsalazine      |                      |                                       |                                                                           | Houston et al. (1982)                                                 |
| Metronidazole               | Antibacterial agent  | Cl. perfringens                        | Enzymatic cleavage of ring to N-(2-hydroxyethyl)-oxamic acid and acetamide | Kajinami et al. (2005)                                               |
| Levamisole                  | Anthelmintic drug    | Bacteroidetes and Cl. sporogenes       | Enzymatic cleavage of ring to levametabol-I, levametabol-II, and levametabol-III | Gingell and Bridges (1973)                                        |
| Omeprazole                  | Antulcerotics        | Anaerobic bacteria such as Bacteroides strains | Enzymatic reduction to corresponding sulfide metabolites | Kim (2015)                                                        |
| Zonisamide                  | Anticonvulsant       | Cl. sporogenes                         | Enzymatic reduction of benzoisoxazole ring to 2-sulphamoylacetylphenol   | Kim et al. (2000)                                                     |
| Lactulose                   | Laxative drug        | Bifidobacterium bifidum, Lactobacillus acidophilus, Cl. sporogenes | Two enzymatic conversion to lactic and acetic acids                      | Kim et al. (1992, 1998)                                              |
| Sodium salicylate           |                      | Arylsulfate sulfotransferase of Eubacterium rectale | Enzymatic conversion to 4,49-(pyridin-2-ylmethylenedi) diphenoquinone | Kinross et al. (2011) and Kitamura et al. (1997)                      |
| Glycyrrhizin                | Antiviral drug       | Eubacterium. sp. strain GLH            | Enzymatic conversion to 18b-glycyrrhethic acid                            | Kinross et al. (2011) and Kitamura et al. (1997)                      |
| Budleiside                  | Anticaner drug       | α-l-rhamnosidase and β-d-glucosidase of Escherichia sp., Escherichia sp., Enterococcus sp. and Bocillus sp. | Two enzymatic conversion to acacetin                                    | Klaassen and Cui (2015)                                             |
| Ginsenoside Rb1             | Health protection    | Oscillobacter spp., Ruminococcus spp., Holdemania spp., and Sutterella spp. | Enzymatic conversion to 20-O-b-D-glucopyranosyl-20(S)-protopanaxadiol | Klotz (1985) and Koch and Goldman (1979)                              |
| Sennosides                  | Laxative drug        | Reductase and 3-b-D-glucosidase        | Two enzymatic conversion to rhenanthrone                                  | Kourtes et al. (2013)                                               |
| Digoxin                     | Cardiotonics         | Eggerthella lenta possessing a two-gene cytochrome-encoding operon | Enzymatic conversion to dihydrodigoxin and dihydrodigoxigenin           | Lederberg (2000), Lee et al. (2015), and Ley et al. (2005, 2006)        |
| Levodopa                    | Anti-psychochics     | Helicobacter pylori                    | Enzymatic dehydroxylation to m-tyramine or m-dihydroxyphenlacetic acid to sequestering it from brain | Li and Nikaido (2004), Liang et al. (2015) and Lindenbaum et al. (1981) |
| Acetaminophen               | Analgesics           | P-cresol-produce bacteria like Cl. difficile | High baseline microbial metabolite p-cresol compete for clearance by hepatic sulfotransferase and diminish the host's metabolic capacity for Phase II sulfonation of acetaminophen | Niemi et al. (2011) and Okuda et al. (1998)                              |
| Sorivudine                  | Antiviral drug       | Bacteroidetes sp. or enterobacteria (Klebsiella pneumoniae) | Enzymatic conversion to (e)-5-(2-bromovinyl) uracil binding to related enzymes irreversibly to inhibit the metabolism of 5-fluorouracil and incite drug toxicity | Fiddian-Green (2006), Putman et al. (2000) and Qin et al. (2010) |
| Irinotecan                  | Anticaner drug       | β-glucuronidase                        | Hydrolysis of SN-38-G of irinotecan (prodrug) release SN-38 in the intestines which leads to gastrointestinal toxicity | Ramakrishna (2013), Roberts et al. (2013, 2002), and Robertson et al. (1986) |
| Diclofenac                  | Anti-inflammatory    | Anti-inflammatory drug                 | Microbial β-glucuronidase reactive glucuronidated metabolite to cause gastrointestinal toxicity | Rooks and Garrett (2016)                                           |
| Indomethacin                | Anti-inflammatory    | Anti-inflammatory drugs               | Anti-inflammatory drugs                                                  | Rooks and Garrett (2016) and Routy et al. (2018)                      |
| Ketoprofen                  | Anti-inflammatory    | Ketoprofen                             | Anti-inflammatory drugs                                                  | Rooks and Garrett (2016)                                             |
| Statins                     | Hypolipidemics       | Bacteria like Lactobacillus sp. is involved in production of coprostanol | Microbially derived secondary bile acids may compete with simvastatin for hepatic uptake by SLCO1B1101 transporters | Robertson et al. (1986), Sepehr et al. (2009), Shu et al. (1991), and Singh et al. (2016) |
| Nitragepam                  | Hypnotic, sedative, | Nitroreductase                         | Bacterial enzymatic conversion to 7-aminonitragepam, followed by acetylation to 7-acetylanitroreductase in the liver | Takeno et al. (1993), Takeno and Sakai (1991) and Tao et al. (2016) |
|                            | anticonvulsant and anxiolytic drug |                                   |                                                                           |                                                                  |
2.1. Mechanisms of microbial direct action on drug metabolism

2.1.1. Secreting microbial enzymes to transform prodrug molecules

The earliest reported example about prodrug activation by enzymatic activity of intestinal microbiota is the conversion of oral prontosil and neoprontosil into sulfanilamide. Prontosil is the first commercially available antibacterial drug that is lower polar than neoprontosil. This pharmaceutically active metabolite is the reason why the two prodrugs exhibit antibacterial activities in vivo (Gingell and Bridges, 1973), while minimal effect in vitro. Animal experiments using mouse models indicated that azoreductases produced by the colorectal microbiota are in charge of this modification (Gingell et al., 1971). It includes a two-step reaction, the formation of the hydrazo compound, followed by a reductive cleavage of the nitrogen bond (Unsalan et al., 2011). Prontosil is converted to sulfanilamide also in the liver and kidney (Fouts et al., 1957). Moreover, prontosil injected intraperitoneally can experience gut bacterial metabolism by being excreted into the gastrointestinal tract via the bile. However, neoprontosil is excreted via the bile without reduction and absorption in the intestine due to its high polarity. Administration with antibiotics disturbing intestinal microbiota inhibits the activation of oral prontosil and neoprontosil into sulfanilamide in rats (Gingell et al., 1971).

Sulfasalazine is another sulfonamide drug that is metabolically activated by bacterial azoreductase enzymes (Peppercorn and Goldman, 1973). Structurally intact sulfasalazine is scarcely absorbed from the upper intestine, but it undergoes bacterial azo reduction, and releases sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA, mesalazine) in the colon. 5-ASA (Houston et al., 1982), which is active in the colon, is used as an active anti-inflammatory moiety in treating inflammatory bowel disease (IBD), while systemically absorbed SP with its antibacterial and immunomodulatory effects, is considered to be useful in the treatment of rheumatoid arthritis (Mikov et al., 2006). Once sulfapyridine is absorbed in the colon, it may be the cause of side effects of sulfasalazine in some patients, such as rash, headache, and anorexia (Peppercorn and Goldman, 1973). To obviate these adverse effects, balsalazide was synthesized from salicylic acid and 4-aminobenzyl-b-alanine. It can be converted potently into 5-ASA also by intestinal microbiota -secreting azoreductase enzymes in some patients, such as rash, headache, and anorexia (Peppercorn and Goldman, 1973). To obviate these adverse effects, balsalazide was synthesized from salicylic acid and 4-aminobenzyl-b-alanine. It can be converted potently into 5-ASA also by intestinal microbiota -secreting azoreductase enzymes (Chan et al., 1983). Another sulfasalazine analogue, olsalazine, is metabolized into two 5-ASAs by the same mechanism (Wadworth and Fitton, 1991). And as the active metabolites of three prodrugs, 5-ASA and SP further undergo acetylation performed by the gut microbiota in fecal suspensions from rats, guinea pigs, dogs and humans (Klotz, 1985). Notably, 5-ASA is acetylated into N-acetyl-5-aminosalicylic acid by individual bacteria under aerobic and anaerobic conditions in human faecal suspension (Hogezand et al., 1992; Deloménie et al., 2001).

Metronidazole, mainly used to treat trichomoniasis, amoebiasis, and giardiasis (Lofmark et al., 2010), is also a long-established antibacterial agent. In the liver, metronidazole is usually modified by side-chain oxidation and gluturonic acid formation to generate hydrophobic products. However, enzymes from Clostridium perfringens (an anaerobic gut bacterium) metabolize metronidazole into ring-cleavage products, N- (2-hydroxyethyl)-oxamic acid, and acetamide in the gut (Koch and Goldman, 1979). Another anthelmintic drug, levamisole is effective in both humans and animals, especially part active agent in colon cancer. It is metabolized into three thiazole ring-opened metabolites: levametabol-I, levametabol-II, and levametabol-III by human intestinal bacteria, mainly Bacteroidetes and Clostridium spp. under anaerobic conditions (Shu et al., 1991).

In addition, several other drugs metabolized directly by intestinal microbes have been reported. Omeprazole is used to treat gastric ulcer, and can be reduced into corresponding sulfide metabolites by anaerobic bacteria such as Bacteroides strains in vitro conditions. But in fact, this bacterial metabolism is unlikely to occur because oral omeprazole is absorbed well, and does not reach the colon in vivo (Watanabe et al., 1995). Zonisamide, an anticonvulsant clinically used to treat epilepsy, undergoes reduction of benzoisoxazole ring performed by Clostridium sporogenes into 2-sulphamoylacetophenol in vivo (Kitamura et al., 1997). Two laxative drugs, lactulose and sodium picosulfate (Laxoberon), exert their effects through gut microbial metabolism. The former is converted by enzymes of several kinds of intestinal bacteria (Bifidobacterium bifidum, Lactobacillus acidophilus and Clostridium perfringens) into fructose and galactose followed by transformation into lactic and acetic acids. These end products promote the excretion of ammonia and amonia into the feces to relieve hepatic encephalopathy through lowering the pH within the intestine (Elkington et al., 1969; Grundmann, 2010). The latter is metabolized into 4,49-(pyridin-2-ylmethanediyl) diphenol by arylsulfate sulfotransferase of Eubacterium rectale in the colon (Kim and Kobashi, 1986; Kim et al., 1992). However, some anti-allergic drugs, such as diphenhydramine and chlorpheniramine, can cause or aggravate constipation by slowing intestinal peristalsis. This causes the feces to remain in the intestines for a long time and its moisture is excessively lower. Drinking 2500–3000 ml of water per day during medication or changing drugs with new anti-histamines (loratadine and cetirizine) rather than taking laxatives, can improve this difficulty in defecation.

Some Chinese medicine ingredients are also metabolized by the intestinal microbial enzyme into the corresponding absorbable aglycones (Xu et al., 2017), and their prototypes are poorly absorbed in the intestines. Glycyrrhizin is a sweet-tasting compound of the root of Glycyrrhiza glabra and Glycyrrhiza uralensis, and is used to treat hepatitis C in Japan. Eubacterium sp. strain GLH completely metabolizes glycyrrhizin into 18b-glycyrrhetic acid which is absorbed well (Hattori et al., 1983; Kim et al., 2000). a-L-rhamnosidase and b-d-glucoisidase from some bacteria (Escherichia sp., Escherichia sp., Enterococcus sp. and Bacillus sp.) can convert budesonide into cancer-combating acacetin via acacetin-7-glucoside (Tao et al., 2016). As the main ingredient of Panax ginseng, ginsenoside Rb1 is metabolized into bioactive 20-O-b-D-glucopyranosyl-20 (S)-protopanaxadiol (compound K) by Oscillibacter spp., Ruminococcus spp., Holdemania spp., and Suterella spp. (Akao et al., 1998; Kim et al., 2013) Sennosides is converted to 8-glucosyl-rheinanthrone or sennidin monoglucosides by reductase and 3-b-D-glucosidase from the gut microbiota, and the latter is further metabolized into rheinanthrone with purgative property in the distal intestine (Hattori et al., 1980). Several flavonoids glycosides (Kim et al., 1998) rutin, hesperidin, naringin, baicalin, wogonoside, and poncirin and isoflavones (daidzein, genistein (Sepehr et al., 2009) and baicalin (Trih et al., 2010) are metabolized into their respective pharmacologically active metabolites by different intestinal microbiota and enzymes.

2.1.2. Expression of genetic element involved in drug inactivation

Orally administered cardiac glycoside drug digoxin is long known to be metabolized reductively and inactively into dihydrodigoxin and dihydrodigoxigenin by gut Eggerthella lenta. These two metabolites bind incompletely to the Na + + K + -ATPase of the myocardium in some patients (Lindenbaum et al., 1981; Saha et al., 1983; Robertson et al., 1986). These patients account for 10% of total population, and their gut microbiota convert over 40% of oral digoxin into inactive metabolites before its absorption (Li and Jia, 2013). Subsequent researches demonstrate that these people who can reduce digoxin, harbor some strains of E. lentu possessing a two-gene cytochrome-encoding operon, namely the cardiac glycoside reductase (cgr) operon (Haiser et al., 2014). This cgr
operon works by producing a protein-Cgr1-Cgr2 complex that binds to digoxin, and accounts for digoxin’s consequent reduction. And arginine is found to inhibit the reduction of digoxin in this study, so high-protein diet can help improve the efficacy of digoxin in patients who carry cgr + E. lentas (Haiser et al., 2014).

### 2.1.3. Sequestering drugs from the site of action

Normally, orally administered levodopa (L-dopa), as a precursor of dopamine, passes through the blood-brain barrier, and undergoes decarboxylation within the central nervous system (CNS). It can increase the level of dopamine in the CNS to exert its therapeutic effect on Parkinson’s disease. However, one study on incubation of levodopa with rat cecal contents showed that most of the L-dopa was converted to m-tyramine or m-hydroxyphenylacetic acid through gut microbial decarboxylation process, which makes operative L-dopa reduced in the CNS (Goldin et al., 1973). Moreover, people infected with Helicobacter pylori are more common in patients with Parkinson’s disease than in healthy controls. Subsequent studies have revealed that Helicobacter pylori is the major strain involved the bacterial decarboxylation (Pierantozzi et al., 2001; Fiddian-Green, 2006; Hashim et al., 2014).

### 2.1.4. Developing bacterial transporters to change the efficacy of drugs

Bacteria have evolved multidrug transporters rather than highly selective drug-specific transporters to protect themselves from antibiotics and other antibacterials (Li and Nikaio, 2004). Great majority of these multidrug transporters belong to secondary transporters, using transmembrane electrochemical gradients, typically the proton motive force (Putman et al., 2000), to drive drug efflux. The secondary transporters include five subclass transporter families, namely resistance-nodulation-division (RND), the major facilitator superfamily (MFS), the multidrug and toxic compound efflux (MATE), and the small multidrug resistance (SMR) superfamily (Kourtaki et al., 2013). A few belong to another transporters, the ABC transporters, which utilizes the energy released from ATP hydrolysis (Davidson and Chen, 2004). Bacillus subtilis and Lactococcus lactis harbour two types of transporters. Genome of the former encodes for at least 78 ABC transporters, of which BmrA belongs to the ABC family, and Bmr and Bt are listed as the secondary transporter (Torres et al., 2009). Overexpression of any transporter in B. subtilis results in similar decreases in susceptibility to puromycin, chloramphenicol, doxorubicin, and fluoroquinolone antibiotics. However, L. lactis is found to express 40 putative drug transporters by in silico analysis of the genome (Li and Nikaio, 2004). Expression of the mdt (A) gene in L. lactis increased resistance to macrolides, streptogramins, lincosamides, and tetracyclines (Perreten et al., 2001). The presence of membrane protein BbmR in Bifidobacterium breve also increases its resistance to macrolides (Abelardo et al., 2006). These various transporters have an effect on the efficacy of antibacterials.

### 2.2. Mechanisms of gut microbes on affecting host’s capacity of metabolizing therapeutic drugs

#### 2.2.1. Producing microbial metabolites to compete enzymes with drugs

After exerting analgesic and antipyretic effects at the normal dose, acetaminophen is cleared in the form of conjugated metabolites, mainly through two phase II conjugation in the liver, namely glucuronidation and sulfation. However, a small part of excess acetaminophen can be transformed to N-acetyl-p-benzoquinone imine by CYP1A cytochrome (Wacher et al., 2001). N-acetyl-p-benzoquinone imine is further conjugated with hepatic glutathione (GSH) for detoxification. If GSH is absent, excess N-acetyl-p-benzoquinone imine is the cause of hepatotoxicity (Zhang et al., 2017). The pre-dose urinary concentration of a microbially metabolite, p-cresol, is related to the severity of acetaminophen hepatic toxicity (Clayton et al., 2009). As p-cresol and acetaminophen are both substrates for cytosolic sulfotransferases (Gamage et al., 2006), high baseline p-cresol produced by Clostridium difficile and others competes with acetaminophen for binding with the enzyme, leading to changes in the bioavailability of acetaminophen and its metabolites. This change induces the individual variation in acetaminophen metabolism and hepatic toxicity or failure (Larson et al., 2012). Concurrently, the liver damage resulted by acetaminophen administration at night is more severe than that in the morning (Kim and Lee, 1998). In a study with mice, Gong et al. reported that gut microbial metabolite, 1-phenyl-1,2-propanediol mediates diurnal variation of acetaminophen induced hepatotoxicity by exhausting hepatic GSH levels (Gong et al., 2018). However, given the complexity of acetaminophen metabolism and the opposite result of an animal experiment to the above findings (Possamai et al., 2015), the effects of intestinal microbial metabolism on acetaminophen-induced hepatotoxicity require further investigation. Other bacterial metabolites, pyruvic acid and lactic acid, induce dendrite protrusion via GPR31 in intestinal CX3CR1 mononuclear cells to enhance immune response, that may also influence drugs metabolism (Morita et al., 2019). Sorivudine, a Japanese antiviral drug, produces a lethal effect through a mechanism similar to acetaminophen (Okuda et al., 1998). There are 18 acute deaths of patients related to co-administration of sorivudine and 5-fluorouracil, since sorivudine metabolite (e)-5-(2-bromovinyl) uracil is produced by fermentation of the Bacteroidetes sp. (Nakayama et al., 1997) or enterobacteria (Klebsiella pneumoniae) (Ashida et al., 1993). When sorivudine is concomitantly administered with oral 5-fluorouracil produg (an anticancer drug), the metabolite binds to related enzymes irreversibly to inhibit the metabolism of 5-fluorouracil and incite its toxicity.

#### 2.2.2. Promoting enterohepatic recirculation of drugs to metabolize drugs again

In enterohepatic recirculation, drugs entering the gastrointestinal tract are absorbed intestinally by the enterocytes, and subsequently are taken up into the hepatocytes via the portal vein (Roberts et al., 2002). When the drug is secreted into the intestine with bile, the intestinal bacteria can restore the active drug by hydrolysis and reduction or allow it to be reabsorbed to its site of action (Martinez and Amidon, 2013). The metabolism of irinotecan (CPT-11) is a good example of reactivation of drug by intestinal microbiota that has been inactivated in the liver. SN-38 is an intravenous prodrug of the antineoplastic topoisomerase I inhibitor SN-38 for colorectal cancers (Vanhoefer et al., 2001). It is known that irinotecan needs to be hydroyzed by a carboxylesterase in tissues and serum to produce SN-38 (de Jong et al., 2004), an active metabolite exerting its pharmacological effects. SN-38 is conjugated by hepatic UDP-glucuronosyltransferase 1A1 into the inactive SN-38-G (Kajinami et al., 2005). However, SN-38-G experiences biliary secretion into the intestines where it is deconjugated back to SN-38 by β-glucuronidase produced by bacteria, including Escherichia coli, Bacteroides vulgatus, and Clostridium ramosum. This reactivation leads to the dose-limiting adverse side effects, such as delayed diarrhea, weight loss, and suppression of the immune system (Wallace et al., 2010) Fig. 1. In line with SN-38, the enteropathic adverse reactions arise from protracted exposure to carboxylic acid-bearing non-steroidal anti-inflammatory drugs (NSAIDs) including diclofenac, indomethacin and ketoprofen. They are also related to the enterohepatic circulation of glucuronides and caused by reactivation of active metabolites by microbial β-glucuronidase (Saith et al., 2014). Antibiotic treatment or application of inhibitors of microbial β-glucuronidase is reported to reduce the severe side effect inflicted by CPT-11.
(Roberts et al., 2013) and by these NSAIDs, especially indomethacin in animal experiment. A subsequent study demonstrated that the absence of bacterial de-glucuronidation resulted in reduced indomethacin reabsorption into the circulation, with increased elimination and a shortened half-life to follow (Liang et al., 2015).

2.2.3. Affecting bile acid metabolism to modulate systemic drug absorption

Due to their potent competition with 3-hydroxy-3-methylglutaryl-Co A (HMG-CoA) reductase (Davidson and Toth, 2005) to reduce plasma levels of low-density lipoprotein cholesterol (LDL-C) (Postmus et al., 2014); statins are widely prescribed pro-drugs for treating hypercholesterolemia. Previously, great variations among individuals on therapeutic effects of statins are only partly attributed to genetic differences (Mangravite et al., 2006; Verschuren et al., 2012). Subsequent studies demonstrated that gut microbiota are also responsible for statins bioavailability (Kaddurahdaouk et al., 2011). Gut microbiota-derived bile acids and statins share transporters in the gut and liver, such as organic anion transporter SLCO1B1 (Niemi et al., 2011). The gene encoding SLCO1B1 contains a single nucleotide polymorphism, rs4149056, which is associated with plasma concentrations of simvastatin acid and seven bile acids (7α-hydroxycholesterol, cholic acid, taurocholic acid, glycocholic acid, taurochenodeoxycholic acid, lithocholic acid, taurolithocholic acid). The genetic polymorphism may cause competition between simvastatin and bile acids for hepatic uptake by SLCO1B1 transporter (Sayin et al., 2013). Concurrently, it is also shown that incubation of lovastatin with human or rat fecalase preparations produces four metabolites (Wallace et al., 2010), including active β-hydroxy acid metabolite that is known to inhibit HMG-CoA reductase (Yoo et al., 2014). Thus, gut microbiota

Fig. 1. Intestinal microflora participate in the enterohepatic recirculation of CPT-11, causing adverse side effects. (a) In tissues and serum, irinotecan is hydrolyzed by a carboxylesterase to produce SN-38, an active metabolite exerting its pharmacological effects. (b) In the liver, SN-38 is conjugated by UDP-glucuronosyltransferase 1A1 into the inactive SN-38-G. (c) SN-38-G experiences biliary secretion into the intestine. In the intestine, SN-38-G is deconjugated back to SN-38 by β-glucuronidase produced by bacteria, including Escherichia coli, Bacteroides vulgatus, and Clostridium ramosum.

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can influence the pharmacokinetics of simvastatin by competing with statins for absorption or co-metabolizing statins.

### 2.2.4. Affect the “components” of the host about drug metabolism

Emerging evidence indicates that the imbalance of intestinal microbiota (germ-free mice and antibiotics-treated mice) alters expression levels of multiple drug-metabolizing enzymes and transporters in liver and kidney of mice, where most drugs are cleared. Among them, the decrease in CYP2B6 activity in human liver results in significant inter-individual differences in the metabolic activities towards cyclophosphamide, efavirenz and bupropion. Decreased protein expression level of BCRP also changes the pharmacokinetics of anticancer drugs, such as mitoxantrone and topotecan (Kuno et al., 2016). But, their related microbiota are unknown.

### 2.2.5. Producing a unique metabolite inducing hepatic further metabolism

The involvement of gut microbiota in the metabolism of nitrazepam, a hypnotic, sedative, anticonvulsant and anxiolytic drug, into its teratogenicity was demonstrated in experiments with rats (Takeno and Sakai, 1991). Nitrazepam is transformed to 7-amino nitrazepam by nitroreductase from intestinal microbiota, followed by acetylation to 7-acetylaminonitrazepam in the liver (Takeno et al., 1993). Although reductive metabolism in rat liver is less potent than in gastrointestinal tract (Rafi, 1997), two metabolites are both teratogenic. The involvement of the gut microbiota in metabolism of above-mentioned drugs has been further demonstrated by studies in germ-free or antibiotic-treated rats. Metronidazole is the only drug with unchanged metabolism in these studies (Pierce et al., 2014). It is initially presumed that the specie involved in the metabolism of metronidazole is not sensitive to antibiotics. Currently, metabolisms of many other drugs seem to also be related to the intestinal microbiota, but the specific bacteria or bacterial enzymes involved are unclear, and need to be further explored. Additionally, the mechanism by which the known intestinal microbiota affects drugs pharmacokinetics is by no means limited to the known. For example, the precise manner in which Faecalibacterium prausnitzii dictates tacrolimus dosing requirements is unknown (Lee et al., 2015).

### 3. The possible impact of gut microbiota on immune checkpoint inhibitors

In addition to the important role of gut microbiota in preventing disease, regulating immunity and metabolizing chemical or Chinese herbal medicine, these microbes have been shown to affect the immunomodulatory effect and side effect of immunotherapy. In 2015, two researches first confirmed that the composition of intestinal microbes determines the effectiveness of cancer immunotherapy in mice. Namely, the effect of the antibody of Ipilimumab targeting the immune checkpoint blocker CTLA-4 required Bacteroides thetaiotaomicron and Bacteroides Fragilis (Vétizou et al., 2015), and Bifidobacterium spp. favored the efficacy of anti-programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) monoclonal antibody (Sivan et al., 2015).

Subsequent studies on human further confirmed the close relationship between intestinal bacteria and immunotherapy. Zivogel and his team revealed that gut microbiota, especially Akkermansia muciniphila which enhances the recruitment of CCR9 + CD43 + CD4+ T lymphocytes into tumor beds, has an impact on clinical benefit of immune checkpoint inhibitors targeting the PD-1/PD-L1 in patients with cancer (Routy et al., 2018). Another two studies published in the same issue of Science on melanoma patients treated with PD-1 blockade respectively show that beneficial strains are more abundant in the guts of blockade-responding patients. The bacteria involved in the former are bacteria of the Ruminococcaceae family (Gopalakrishnan et al., 2018), while the latter are Bifidobacterium longum, Collinsella aerofaciens and Enterococcus faecium (Matson et al., 2018). These gut microbes may induce dendritic cells to release some cytokines which recruit more effector T cells to focus around the tumor. Gut microbiota may also be the reason why immune checkpoint inhibitors have clinical benefits for only 20–30% of treated cancer patients (Botticelli et al., 2016; Wang et al., 2018a). Therefore, appropriate strains can assist the immune system in responding to these treatments. Manipulating the gut microbiota is expected to be a new strategy to increase the activity of immunotherapeutics, and may also be used to reduce their side effects. Three related results were obtained with germ-free mice or antibiotics-treated mice, which reported improved response to anti-PD-1 therapy in mice given feces from responsive cancer patients. The mice receiving fecal transplants from responding patients also showed enhanced systemic and antineoplastic immunity (Gopalakrishnan et al., 2018; Matson et al., 2018; Routy et al., 2018). Notably, significant and rapid cure of refractory immune checkpoint inhibitors (ICI)-associated colitis has been derived from fecal microbiota transplantation (FMT) with changes in gut microbiial composition and T cell composition within the colonic mucosa of two cancer patients (Wang et al., 2018b). FMT benefits not only immunotherapy, but also other cancer treatments and reduces associated side effects, such as radiotherapy (Cui et al., 2017) and taking pacitaxel (Castelli et al., 2018). The list of immunological checkpoint inhibitors (Ayelet et al., 2015; Frankel et al., 2017; Wargo et al., 2017; Chapat et al., 2017) that are affected by the specific gut microbiota is shown in Table 2.

### 4. Existing limitations

It is known that intestinal microbes can modulate drug metabolism in several proposed ways including direct enzymatic effects on transform of drug molecules and affecting the host function to interfere with drug metabolism, such as changes in drug metabolizing enzymes, metabolic type and basal metabolism of host. Such studies accelerate the understanding of gut microbes and the correct application of drugs, but the animal models, germ-free mice and antibiotics-treated mice, have some limitations. Germ-free mice are abnormal mice with enlarged caecum, reduced villous thickness and villous capillary networks (Tagger and Maurice, 2016), and they are not equivalent to normal mice without gut flora. In terms of metabolism, germ-free mice consume more calories and secrete more lipids (Wostmann et al., 1983), which may make them more susceptible to low-grade inflammation. And germ-free mice show underdeveloped intestinal-associated lymphoid tissues, reduced production of secretory IgA and regulatory T cell, and imbalanced TH1–TH2 cell in immunity (Rooks and Garrett, 2016). Germ-free mice also have abnormalities in signal transduction. Endocrine small molecules, such as dopamine which is critical to the development of the enteric nervous system, in germ-free mice are decreased (Borre et al., 2014). Similarly, antibiotics alterate not only intestinal microbiota but also the function of other organs in antibiotic-treated mice. Most experiments in vitro involve the incubation of drugs with bacterial suspensions
prevented from rat or human cecal contents. Since the suspensions mix different bacteria that are in different positions and cannot contact each other in the gut in vivo, which may influence the order of different reactions, and some strains even cannot survive in vitro. So we can see that these operations do not accurately reflect the metabolism of the drug in vivo, although they allow us to discover the microbiota-mediated metabolites. Concurrently, the potential difference between in vivo and in vitro models of the gut microbiota should be taken into consideration when analyzing studies of drugs biotransformation, as some drugs are not metabolized in vivo but metabolized in vitro. These drugs are effectively absorbed before reaching the lower part of the intestine where containing more kinds of bacteria. Taken together, even if these models have certain reference value, it is very necessary for us to find more suitable models.

Given the wide variety of drugs affected by intestinal microbes, it is pivotal for personalized medicine to explore additional drugs affected by gut microbiota and their involved strains for further making mechanism clear. There is an overlap between different classes of drug metabolizing pathways. Thus, when exploring the unknown metabolic pathways of other drugs, it is conceivable that these pathways may be partially similar to the known pathways described above. Sometimes drug treatment outcomes of two individuals are different, although they all contain the related microorganisms and enzymes. Other factors may be involved in, e.g. microbial-induced host gene quantitative or qualitative changes, especially at the level of epigenetic regulation. Concurrently, although intestinal microbial regulation of microRNA in the brain (Hoban et al., 2017) and effect on drug metabolism enzymes and transporters in host liver and kidney (Kuno et al., 2016) have been confirmed, the mechanisms by which microbes cause these variations and the optimal treatments are in their initial stages.

5. Future directions

On the basis of existing research results, we propose these promising research points: (I) At present, there are only over 30 drugs that are known to be closely related to the intestinal microbiota, and we need to further clarify other drugs affected by the microbiota, and the mechanism involved. (II) In addition to germ-free mice and antibiotic-treated mice, studies on microbiota and drug metabolism require more scientific animal models. (III) There may be not only one kind strain involved in when intestinal microbiota affects drug metabolism and side effects, but may several “related” microbiota implicated in, or an equilibrium state of the whole microbiota related to. This conclusion may be different depending on the specific drugs taken. (IV) It is necessary to strengthen the in vivo study on the interaction of microbiota, host intestinal epithelial cells and drugs in the intestinal microenvironment, especially the microbical regulation of genes related to drug metabolism in the host. (V) By analyzing the relationship between intestinal microbiota traits (diversity, composition, metabolites) and drug metabolism, it is expected to develop a estimating system for bacterial population (before administration) related to drug use efficiency and side effects. (VI) When interfering with intestinal microbes, it is warranted to choose the right means based on the principle of completeness to restore intestinal microbiota balance, rather than only supplement what is short. Considering that FMT has made progress in the treatment of intestinal diseases (He et al., 2017; He et al., 2017; Johnsen et al., 2018), we can expect whether transplantation of normal human microbiota to patients with abnormal drug metabolism also has therapeutic effects. But, special attention should be paid during the operation to prevent infection which may endanger the patient’s life. (VII) On the basis of clarifying the particular microbiota that affects the bioavailability of a drug, it may be considered to explore the strategy of taking the drug in combination with this related microbiota, but to ensure that this microbiota survives after entering the intestine without disturbing the overall microbiota balance (avoiding causing new problems), and acts on the same part of the intestinal tract with the drug. (VIII) Similarly, other means of regulating the microbiota of a patient may also be considered, such as co-administration of drug and prebiotics or symbiotics that are beneficial to the microbiota. It is necessary to explore new microbial inhibitors, microbial enzyme inhibitors or microbiota-derived molecules (active metabolites or microbial gene products.

Table 2

| Target          | Specific agents | Clinical application | Bacterial Species involved | Mechanism                                                                 | Ref.                                      |
|-----------------|-----------------|----------------------|----------------------------|--------------------------------------------------------------------------|-------------------------------------------|
| PD-1            | Pembrolizumab   | Melanoma             | Dorea formicigenerans      | Unmentioned                                                              | Watanabe et al. (1995)                    |
| PD-1            | Unidentified    | Melanoma             | Clostridales               | increasing tumor immune infiltrates and density of CD8+ T cells          | Westmann et al. (1983)                    |
| PD-1            | Unidentified    | Melanoma             | Ruminococcaceae           | increasing density of CD8+ T cells                                       | Wadsworth and Fitton (1991)               |
| PD-1            | Unidentified    | Melanoma             | Bifidobacterium longum,   | improving tumor control, augmenting T cell responses and enhancing efficacy of anti-PD-L1 therapy | Vanhoefer et al. (2001)                   |
|                 |                 |                      | Collinella aerofaciens,    |                                                                          |                                           |
|                 |                 |                      | Enterococcus faecium      |                                                                          |                                           |
| PD-L1           | αPD-L1 mAb      | Melanoma             | Some Bifidobacterium species | enhancing CD8(+) T cell priming and accumulation around the tumor         | Wilson and Nicholson (2017)              |
|                 |                 |                      |                            |                                                                          |                                           |
| CTLA-4          | Ipilimumab      | Melanoma             | Faecalibacterium praunztizii, | reducing proportion of peripheral blood regulatory T cells induced TH1 immune responses in the tumor-draining lymph nodes and promoting the maturation of intratumoral DCs | Wu et al. (2011)                          |
|                 |                 |                      | Dorea formigenerans       |                                                                          |                                           |
|                 |                 |                      | Bacteroides thetaotaomicon,|                                                                          | Trinh et al. (2010)                       |
|                 |                 |                      | Bacteroides fragilis      |                                                                          |                                           |
| PD-1/PD-L1      | Unidentified    | Non-small cell lung cancer, renal cell carcinoma and urothelial carcinoma | Akkermansia muciphilta | Enhancing the recruitment of CCR9 + CXCR3 + CD4 + T lymphocytes into tumor beds | Unsalan et al. (2011)                     |
| CTLA-4 and PD-1 | Ipilimumab       | Melanoma             | Fecalibacterium praunztizii, | Unmentioned                                                              | Watanabe et al. (1995)                    |
|                 | plus nivolumab  |                      | Bacteroides thetaotaomicon,|                                                                          |                                           |
|                 |                 |                      | Holdemania filiformis     |                                                                          |                                           |

mAb monoclonal antibody; TH1 T helper 1; DCs dendritic cells.
which are equally beneficial to the drug, when the microbiota is not conducive to the metabolism of the drug. (IX) Co-administration of sorivudine and 5-fluorouracil is known to incite acute deaths. More drug-drug interactions involved in intestinal microbiota should be investigated. Answering these questions is conducive to the continued research of drugs and improving individualized medicine.

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Declaration of interest statement

Declarations of interest: none.

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