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DRUG METABOLISM & DISPOSITION

Special Section on Drug Metabolism and the Microbiome—Minireview

Investigation of Host–Gut Microbiota Modulation of Therapeutic Outcome

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ABSTRACT

A broader understanding of factors underlying interindividual variation in pharmacotherapy is important for our pursuit of "personalized medicine." Based on knowledge gleaned from the investigation of human genetics, drug-metabolizing enzymes, and transporters, clinicians and pharmacists are able to tailor pharmacotherapies according to the genotype of patients. However, human host factors only form part of the equation that accounts for heterogeneity in therapeutic outcome. Notably, the gut microbiota possesses wide-ranging metabolic activities that expand the metabolic functions of the human host beyond that encoded by the human genome. In this review, we first illustrate the mechanisms in which gut microbes modulate pharmacokinetics and therapeutic outcome. Second, we discuss the application of metabonomics in deciphering the complex host–gut microbiota interaction in pharmacotherapy. Third, we highlight an integrative approach with particular mention of the investigation of gut microbiota using culture-based and culture-independent techniques to complement the investigation of the host–gut microbiota axes in pharmaceutical research.

Introduction

Our unique age, sex, genetic makeup, nutritional state, disease condition, and environmental exposure influence therapeutic outcomes. The recognition of interindividual differences in drug response has thus spawned the move toward "personalized medicine" (Mancinelli et al., 2000; Ginsburg and McCarthy, 2001; Woodcock, 2007; Hamburg and Collins, 2010). The ability to truly provide personalized medical care is dependent on mechanistic knowledge underlying heterogeneity in drug responses. With the emergence of "omics" technologies, there has been a paradigm shift toward investigating diseases and drug therapies using the omics-based systems biology approach (Zhou et al., 2008; Lum et al., 2009; Bates, 2010; Chan and Ginsburg, 2011). The recommendation by the U.S. Food and Drug Administration to genotype patients prior to treatment with trastuzumab, clopidogrel, carbamazepine, and irinotecan due to the differential efficacy and toxicity of these drugs in stratified patient cohorts demonstrated the value of pharmacogenomics in predicting drug responses (Khoury et al., 2009; Leckband et al., 2013). Nonetheless, host genetics alone does not explain all variations in pharmacotherapy (Nebert et al., 2003).

In fact, human beings have been coined as "superorganisms," since our complex systems biology is dictated by two sets of genomes—the genetically inherited human genome and the environmentally acquired microbiome (Lederberg, 2000). It has been estimated that there are approximately 10^{12} parenchymal cells in humans (excluding blood cells and neurons) and 10^{12} bacteria on the skin, 10^{10} in the mouth, and 10^{14} in the gut (Kumar et al., 2013). Considering the 10-fold higher proportion of microbial to human cells, the extragenomic influence by the microbiome on systems biology should not be underestimated (Savage, 1977; Holmes et al., 2012; Nicholson et al., 2012). Indeed, accumulating evidence revealed the diverse effects of the microbiome on human health, including nutrition, physiology, and host metabolism (Nicholson et al., 2012; Guinane and Cotter, 2013). Dysbiosis of the microbiome has also been linked to pathologic conditions such as inflammatory bowel disease (Morgan et al., 2012; Kostic et al., 2014), diabetes (Qin et al., 2012), obesity (Turnbaugh et al., 2006, 2009; Sweeney and Morton, 2013), and autism (Kang et al., 2013).

From an anatomic perspective, the gut microbiota forms an important external "organ" within the gastrointestinal ecosystem, comprising more than 400 different species of bacteria (Hao and Lee, 2004; Qin et al., 2010). Notably, these intestinal bacteria contribute about 1.5 kg of the human body weight (Toivanen et al., 2001), comparable to the weight of major human organs such as the liver (approximately 1.5 kg) and brain (approximately 1.4 kg) and exceeds that of the lung (approximately 0.84 kg), kidney (approximately 0.27 kg), and spleen (approximately 0.14 kg) (Molina and DiMaio, 2012). Importantly, the gut microbiota performs functional activities not encoded by the host genome. For example, desert woodrats living in

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ABBREVIATIONS: P450, cytochrome P450; qPCR, quantitative polymerase chain reaction; rRNA, ribosomal ribonucleic acid; SN-38, 7-ethyl-10-hydroxy-camptothecin; SPF, specific pathogen-free.

the Mojave desert in the United States were found to harbor gut microbiota that facilitates their dietary consumption of highly toxic creosote bush, which invaded their habitat 17,000 years ago (Karasov, 1989). The leaves of the creosote bush are covered with a phenolic-rich resin that is largely made up of nordihydroguaiaretic acid, which is known to induce kidney cysts and liver damage in laboratory rodents (Lambert et al., 2002; Arteaga et al., 2005). Kohl et al. (2014) demonstrated that the distinct gut microbiota communities residing in Mojave woodrats confer tolerance to the creosote plant toxins by having higher abundance of microbes with genes that facilitate metabolism and detoxification of the aromatic toxic compounds, compared with the naïve woodrats lacking similar ecological and evolutionary experience with creosote. This example highlighted how microbial adaptation expands the host's enzymatic repertoire and confers the host with competitive advantage through access to nutrients that are otherwise toxic to other competitors incapable of performing microbial detoxification (Dearing et al., 2005).

Similarly, the metabolic crosstalk between the host and gut microbiota modulates the pharmacokinetics and pharmacodynamics of drugs (Sousa et al., 2008; Holmes et al., 2012; Haiser and Turnbaugh, 2013; Li and Jia, 2013; Carmody and Turnbaugh, 2014). In this review, we first offer an overview of how pharmacokinetics and therapeutic outcomes are affected by a number of key established host–gut microbiota interactions. Second, we discuss the application of metabonomics in deciphering the complex host–gut microbiota interaction underlying interindividual variation in therapeutic outcome. Third, we propose an integrative approach to investigate the gut microbiota, with a special focus on culture-based and culture-independent techniques.

Host–Gut Microbiota Modulation of Pharmacokinetics and Therapeutic Outcome

The Liver in Pharmacokinetic and Therapeutic Outcome. To understand pharmacokinetic and therapeutic outcome, pharmaceutical scientists have placed much attention on investigating the host (e.g., species, gene expression, genetic polymorphism, disease, sex, and age), drug (e.g., chemical structure, dosage, and frequency of administration), and other xenocompounds (e.g., diet, supplements, and other concomitant drugs), rather than the gut microbiota (Wilson and Nicholson, 2009). The liver has received special attention, because it is a major organ that is responsible for metabolizing xenobiotics . The liver plays a central role in the biotransformation of drugs and is equipped with a range of metabolizing enzymes and transporters necessary for its function. In phase I functionalization reactions (e.g., oxidation, reduction, and hydrolysis), polar functional groups are introduced to nonpolar molecules. Cytochrome P450 (P450) is a major class of drug-metabolizing enzymes in the liver that are responsible for phase I metabolism (Wrighton and Stevens, 1992; Spatzenegger and Jaeger, 1995; Iyanagi, 2007). Phase II enzymes (e.g., UDP-glucuronosyltransferase, sulfotransferases, or glutathione-*S*-transferases) catalyze conjugation reactions that add polar moieties such as glucuronic acid, sulfate, or glutathione to the functional groups (Iyanagi, 2007). These processes facilitate the clearance of the xenobiotics via the urinary or biliary route with the aid of transporters or efflux pumps. Considering the key roles of the hepatic metabolizing enzymes and transporters in pharmacokinetics, factors such as induction, inhibition, and genetic polymorphism that lead to alterations in the expression and functional activities of these enzymes and transporters, may contribute to interindividual variation in pharmacokinetics and therapeutic outcome (Park and Breckenridge, 1981; Park et al., 1996; Ingelman-Sundberg et al., 1999; Ando et al., 2000; Shenfield, 2004; Bosch et al., 2006; Guengerich, 2006; Ieiri et al., 2006; Kerb, 2006; Tomalik-Scharte et al., 2008). However, these factors do not account for all forms of heterogeneity among individuals.

Gut Microbiota Modulates Pharmacokinetics and Therapeutic Outcome. In recent years, interest in elucidating the roles of the gut microbiota on pharmacokinetics and therapeutic outcomes has rekindled. The gut microbiota is known to possess a diverse range of metabolic activities that are capable of modulating the fate of an administered drug and its therapeutic outcome (Scheline, 1968; Nicholson et al., 2005; Sousa et al., 2008; Clayton et al., 2009; Haiser and Turnbaugh, 2012; Haiser et al., 2013). Scheline (1973) even proposed that the gut microbiota has the metabolic potential at least equivalent to the liver.

Mechanistically, the gut microbiota is known to affect pharmacokinetics by partaking in the direct metabolism of xenobiotics or through its indirect interaction with the host enzymatic system (Scheline, 1968; Nicholson et al., 2005; Sousa et al., 2008; Clayton et al., 2009; Haiser and Turnbaugh, 2012; Haiser et al., 2013). The indirect interaction is facilitated through the production of microbial or mammalian-microbial cometabolites that compete for metabolism of xenobiotics or act as signaling molecules that influence the host gene expression. Such interactions may complement or oppose the host's enzymatic activity, culminating in wide-ranging therapeutic consequences. The following section illustrates the roles of the gut microbiota in modulating pharmacokinetic processes and the therapeutic implications. The list of affected drugs is presented in Table 1 (Sousa et al., 2008; Clayton et al., 2009; Kaddurah-Daouk et al., 2011; Haiser and Turnbaugh, 2013; Saitta et al., 2014).

Absorption. The efficacy of statins to control hypercholesterolemia and reduce the risk of cardiovascular disease exhibits great variation among individuals that can only be partly explained by genetic differences (Verschuren et al., 2012). Simvastatin is an inactive prodrug that is widely prescribed for lowering cholesterol levels. Using metabonomics, Kaddurah-Daouk et al. (2011) performed a targeted analysis of a panel of metabolites in the cholesterol synthesis, dietary sterol absorption, and bile acid formation pathways in the plasma of 100 individuals to determine metabolic phenotypes (metabotypes) predictive of variation in cholesterol-lowering efficacy of statins. In their study, the pretreatment concentrations of several primary and secondary bile acids were found to correlate with the on-treatment plasma simvastatin acid (active metabolite) levels. In addition, an association in the plasma levels of simvastatin acid and seven bile acids with a single nucleotide polymorphism, rs4149056, in the gene solute carrier organic anion transporter family, member 1B1 (SCLO1B1) was uncovered. Bile acids and statins share transporters such as organic anion transporting polypeptide 1B1 (OATP1B1) in the intestine and liver (Niemi et al., 2011). The authors postulated that genetic polymorphism of SLCO1B1 might have limited the transport of these substrates, possibly through competition between simvastatin and bile acids for hepatic uptake by the OATP1B1 transporter. Such competition may hence influence the pharmacokinetics, efficacy, and toxicity (e.g., myopathy) of simvastatin. Other drugs that are substrates of the same class of transporters may potentially be subjected to similar interaction with the bile acids. Considering the intricate role of bile acid in drug transport, the gut microbiota may potentially modulate systemic drug absorption through affecting bile acid metabolism (Macdonald et al., 1983; Dawson, 2011; Sayin et al., 2013).

Disposition. In the liver, the metabolism of xenobiotics comprises oxidative and conjugative reactions. On the other hand, it is notable that compared with the metabolic activities of the host, the reactions associated with the gut microbiota are primarily reductive reactions and hydrolytic cleavage of conjugates such as glucuronides and sulfate conjugates that have been secreted via the bile into the gastrointestinal tract (Table 1) (Scheline, 1973; Goldman, 1978; Rowland, 1988). As such, the gut microbiota can generate metabolites that are otherwise not produced by the host.

TABLE 1

Drugs whose pharmacokinetics or therapeutic outcomes are mediated by gut microbiota

Reaction	Drug (Reference)	Bacteria or Its Enzymes	Consequences of Gut Microbiota Metabolism on Drug Pharmacokinetics and Therapeutic Outcomes
Reduction	Prontosil (Gingell et al., 1971)	Azoreductase enzymes	Activation of azo-bond containing prodrug to sulfanilamide
	Neoprontosil (Gingell et al., 1971)		
	Sulfasalazine (Peppercorn and Goldman, 1972)		Activation of azo-bond containing prodrug to 5-aminosalicylic acid
	Balsalazide (Chan et al., 1983)		
	Olsalazine (Wadworth and Fitton, 1991)		
	Nitrazepam (Rafii et al., 1997)	Nitroreductase	Cometabolism of nitrazepam produces 7-acetylaminonitrazepam responsible for teratogenic activity: step 1, nitroreduction of nitrazepam to 7-aminonitrazepam by gut microbiota; and step 2, 7-aminonitrazepam is converted to 7-acetylaminonitrazepam in the liver
	Clonazepam (Elmer and Remmel, 1984)	Not reported	Nearly complete reduction to 7-aminoclonazepam
	Misonidazole (Koch et al., 1980)	Not reported	Reduction to 1-(2-aminoimidazol-1-yl)-3-methoxypropan-2-ol
	Omeprazole (Watanabe et al., 1995)	Not reported	In vitro reduction by gut microbiota to omeprazole sulfide metabolite. However, there was no alteration of oral in vivo pharmacokinetics because omeprazole is fully absorbed before reaching the hindgut
	Sulfinpyrazone (Strong et al., 1987)	Not reported	Reduction to sulfinpyrazone sulfide metabolite (solely by gut microbiota)
	Sulindac (Strong et al., 1987)	Not reported	Reduction to sulindac sulfide metabolite
	Digoxin (Lindenbaum et al., 1981; Haiser et al., 2013)	*Eggerthella lenta*	Reduction to inactive metabolites (e.g., dihydrodigoxin or dihydrodigoxigenin) by gut microbiota reduce therapeutic efficacy
	Zonisamide (Kitamura et al., 1997)	*Clostridium sporogenes*	Reduction to 2-sulphamoylacetylphenol
	Metronidazole (Koch et al., 1979)	*Clostridium perfringens*	Reduction to *N*-(2-hydroxyethyl)-oxamic acid and acetamide
Hydrolysis	Lactulose (Sahota et al., 1982)	*Lactobacillus*, *Bacteroides*, and *Clostridium*	Therapeutic activity depends on its metabolism by intestinal bacteria to form lactic and acetic acids
	Sorivudine (Okuda et al., 1998)	*Bacteroides* species (e.g., *Bacteroides eggerthii* and *Bacteroides vulgatus*)	A major metabolite of sorivudine, (*E*)-5-(2-bromovinyl) uracil, generated from microbial metabolism of the drug was found to inactivate a key hepatic enzyme involved in the metabolism of 5-fluorouracil. Coadministration of sorivudine and 5-fluorouracil resulted in drug interactions that led to death
Deconjugation of drugs excreted in bile as inactive conjugates	Digitoxin (Volp and Lage, 1978)	β-glucuronidase	Hydrolysis of glucuronide
	Indomethacin (Saitta et al., 2014)		Hydrolysis of glucuronide of indomethacin release the aglycone which leads to gastrointestinal toxicity
	Morphine (Walsh and Levine, 1975)		Hydrolysis of glucuronide
	Irinotecan (Roberts et al., 2013)		Hydrolysis of SN-38 glucuronide of irinotecan (prodrug) release SN-38 in the intestines, which leads to gastrointestinal toxicity
Removal of succinate group	Succinylsulfathiazole (Sousa et al., 2008)	Not reported	Activation of prodrug to sulfathiazole
Dehydroxylation	L-Dopa (Goldin et al., 1973)	Not reported	Alteration of L-dopa pharmacokinetics by gut microbiota metabolism to form *m*-tyramine and *m*-hydroxyphenylacetic acid
Acetylation	5-Aminosalicylic acid (Dull et al., 1987; Deloménie et al., 2001)	*N*-acetyltransferase	Acetylation to form acetylated 5-aminosalicylic acid
Deacetylation	Phenacetin (Smith and Griffiths, 1974)	Not reported	Formation of *p*-phenetidin from deacetylation reaction is correlated with toxicities such as methemoglobinemia and nephritis
Cleavage of *N*-oxide bond	Ranitidine (Basit and Lacey, 2001)	Not reported	Susceptible to *N*-oxide bond cleavage by gut bacteria
	Nizatidine (Basit et al., 2002)	Not reported	Susceptible to *N*-oxide bond cleavage by gut bacteria
Proteolysis	Insulin (Tozaki et al., 1997)	Not reported	Susceptible to proteolysis
	Calcitonin (Tozaki et al., 1997)	Not reported	Susceptible to proteolysis
Denitration	Glyceryl trinitrate (Abushamat, 1993; Sousa et al., 2008)	Not reported	Generate glyceryl-1,3-dinitrate, glyceryl-1,2-dinitrate, glyceryl-1-mononitrate, and glyceryl-2-mononitrate
	Isosorbide dinitrate (Sousa et al., 2008)	Not reported	Generate isomeric mononitrates and isosorbide
Amine formation and hydrolysis of amide linkage	Chloramphenicol (Holt, 1967)	Not reported	Metabolized to metabolites such as *p*-aminophenyl-2-amino-1,3-propanediol. Aplasia of the marrow, the most serious complication of chloramphenicol, has been proposed to be attributable to the activity of the intestinal microbiota

(*continued*)

TABLE 1—*Continued*

Reaction	Drug (Reference)	Bacteria or Its Enzymes	Consequences of Gut Microbiota Metabolism on Drug Pharmacokinetics and Therapeutic Outcomes
Thiazole ring-opening	Levamisole (Shu et al., 1991)	*Bacteroides* and *Clostridium* species	Generate levametabol-I, levametabol-I, and levametabol-III metabolites
Isoxazole scission	Risperidone (Meuldermans et al., 1994)	Not reported	Scission of the isoxazole in the benzisoxazole ring system of risperidone is a major metabolic pathway contributed by the gut microbiota
N-Demethylation	Methamphetamine (Caldwell and Hawksworth, 1973)	Not reported	Converted to amphetamine by gut microbiota. May be inconsequential to humans since the parent drug is quite efficiently absorbed in the upper gastrointestinal tract
Competition of microbial metabolite for phase II drug clearance	Acetaminophen (Clayton et al., 2009)	Bacteria like *Clostridium difficile* are *p*-cresol producers	High predose levels of microbial metabolite *p*-cresol compete for clearance by hepatic sulfotransferase and diminish the host's metabolic capacity for phase II sulfonation of acetaminophen
Competition of microbial metabolite for hepatic uptake of drug	Simvastatin (Kaddurah-Daouk et al., 2011)	Bacteria like *Lactobacillus* are involved in production of coprostanol	Microbially derived secondary bile acids may compete with simvastatin for hepatic uptake by SLCO1B1 transporters, thereby affecting the pharmacokinetics and pharmacodynamics of simvastatin, and increasing the risk of myopathy

Nitrazepam, a benzodiazepine drug, has been reported to induce teratogenicity (Takeno and Sakai, 1991). Incubation of nitrazepam with bacterial suspensions prepared from rat cecal contents resulted in extensive reduction to 7-aminonitrazepam, which in turn yielded 7-acetylaminonitrazepam via hepatic acetylation. These metabolites were reported to cause fetal malformation. Suppression of the nitroreductase activity of the gut microbiota by antibiotics before the administration of nitrazepam significantly decreased the urinary and fecal excretion of the two reduced metabolites from 30% to 2% and markedly reduced the incidence of teratogenicity. This underscores the roles of gut microbiota in nitrazepam-induced teratogenicity (Takeno and Sakai, 1991).

Lovastatin is a lactone prodrug that is used in the treatment of hypercholesterolemia. In a recent study by Yoo et al. (2014), incubation of lovastatin with human and rat fecal preparations yielded the active β-hydroxy acid metabolite that is known to inhibit 3-hydroxy-3-methylglutaryl CoA reductase. This suggested the role of gut microbes in activating lovastatin for eliciting its pharmacological effects. The administration of lovastatin to antibiotic-treated rats resulted in a reduced systemic exposure to and an approximately 60% lower fecal production of the active β-hydroxy acid metabolite (Yoo et al., 2014). The decreased bioavailability of the active metabolite as a result of antibiotic consumption diminishes the therapeutic efficacy of lovastatin for cholesterol control. In a wider context, this work illustrated that drug–drug interaction can occur between antibiotics and drugs that are subjected to direct microbial metabolism.

In enterohepatic recirculation, a solute entering the gastrointestinal tract is absorbed intestinally by the enterocytes; it is then taken up into the hepatocytes via the portal vein and is biliary secreted into the intestines, where it can undergo further intestinal reabsorption to be channeled back to the systemic circulation (Roberts et al., 2002). Drugs subjected to enterohepatic recirculation are often characterized by multiple C_{max} peaks, a longer apparent half-life in plasma concentration-time profile, and a larger apparent volume of distribution (Roberts et al., 2002). Examples of drugs subjected to enterohepatic recirculation include irinotecan (Mathijssen et al., 2001), morphine (Walsh and Levine, 1975), and indomethacin (Duggan et al., 1975). Enterohepatic recirculation is often associated with hepatic conjugation of the parent drug and intestinal deconjugation of the metabolite. Irinotecan is a prodrug that is administered intravenously as a first-line therapy for colorectal cancers (Pommier, 2006). However, the utilization of irinotecan in chemotherapy is affected by its dose-limiting gastrointestinal toxicity, which is characterized by severe diarrhea in patients (Rothenberg et al., 1996). Being a prodrug, irinotecan is known to require hydrolysis by carboxylesterase in tissue and serum to generate the active metabolite, 7-ethyl-10-hydroxy-camptothecin (SN-38), for its pharmacologic effect (Mathijssen et al., 2001). The active SN-38 is subjected to phase II glucuronidation by hepatic UDP-glucuronosyltransferase 1A1 to form the inactive SN-38G, which is biliary secreted into the intestines (Mathijssen et al., 2001). Bacteria are reported to be the main contributor of intestinal source of β-glucuronidase enzymes for deglucuronidation (Rod and Midtvedt, 1977; Oleson and Court, 2008). Indeed, it has been discovered that the hydrolysis of SN-38G to SN-38 by microbial β-glucuronidase is responsible for the undesirable side effect of severe diarrhea (Takasuna et al., 1996; Mathijssen et al., 2001). The manipulation of the microbial β-glucuronidase activity with β-glucuronidase inhibitors and antibiotics has been shown to prevent the production of toxic SN-38 metabolites and protect the intestines of mice against injury (Takasuna et al., 1996; Wallace et al., 2010; Roberts et al., 2013). This underscores the therapeutic potential of targeted manipulation of gut microbes in minimizing the toxicity associated with irinotecan.

Phase II conjugation is a means of drug clearance; hence, the hydrolytic deconjugating activity of the gut microbiota may potentiate the pharmacological or toxicological effects of parent drugs by augmenting their systemic exposure if the aglycones are subjected to enterohepatic recirculation (Scheline, 1973; Goldman, 1978; Rowland, 1988). In a study by Clayton et al. (2009), the team identified that in patients administered with 1 g acetaminophen, individuals that have high predose urinary levels of *p*-cresol sulfate had a low postdose urinary ratio of acetaminophen sulfate to acetaminophen glucuronide. The authors attributed the reduced metabolic clearance of acetaminophen sulfate in implicated individuals to the generation of higher levels of microbially derived *p*-cresol that compete with acetaminophen for phase II sulfonation in the liver (Clayton et al., 2009). Extrapolating this finding, one may hypothesize that the gut microbiota potentially affects the disposition of other drugs, whereby sulfonation is an essential metabolic pathway. In addition, the study by Clayton et al. (2009) illuminated an alternative mechanism of host–gut microbiota interaction mediated via microbial metabolites or cometabolites. This mode of interaction completely bypasses the need for direct contact between the drug and gut microbiota, yet enables the gut microbiota to exert a remote influence on the fate of drug disposition by effecting host metabotypic changes that in turn modulate the host's enzymatic activities. In light of the extensive influence of the gut microbiota on the host metabotype (Li et al., 2008; Zheng et al., 2011),

it is believed that the work by Clayton et al. sets a new direction to investigate the pervasive influence of gut microbiota on the pharmacokinetics of drugs.

From the above examples, it is clear that the gut microbiota possesses diverse metabolic capabilities that augment or reduce the metabolic status of the host and produce active, inactive, or toxic metabolites, some of which are not synthesized endogenously. Collectively, the current evidence emphasizes the importance of the gut microbiota in pharmaceutical research and personalized medicine.

Elucidating Host–Gut Microbiota Interactions: A Need (Not Want). Approximately 3936 molecular entities have been approved for human use by major markets worldwide, including the United States (Huang et al., 2011). Despite the vast metabolic potential of the gut microbiota, it has been estimated that only about 40 marketed drugs have been identified as substrates to date (Sousa et al., 2008; Haiser and Turnbaugh, 2013). The relatively few examples related to microbial xenobiotic metabolism are a reflection of our limited knowledge in this field.

Many recent metagenomics studies observed large variation in the gut microbiota composition among individuals across different age groups and populations (Peterson et al., 2009; Turnbaugh et al., 2009; Yatsunenko et al., 2012). Considering the profound metabolic roles exerted by the microbes as described earlier, such variations in gut microbial composition may hold important clinical implications. Digoxin used in treating heart failure is known to possess a narrow therapeutic index and requires therapeutic drug monitoring. It has been long known that digoxin can be reduced and inactivated by gut *Eggerthella lenta* into dihydrodigoxin and dihydrodigoxigenin (Saha et al., 1983; Robertson et al., 1986). Ten percent of the population is known to harbor these enteric bacteria, which metabolize more than 40% of the orally ingested dose of digoxin to inactive metabolites prior to its absorption (Li and Jia, 2013). Coadministration of antibiotics that eradicate the gut bacteria (e.g., erythromycin and tetracycline) has been reported to decrease microbial metabolism of digoxin (Lindenbaum et al., 1981). However, not all patients harboring a high abundance of *E. lenta* will reduce digoxin (Saha et al., 1983). Haiser et al. (2013) recently found that the pharmacokinetics of digoxin is determined by the concerted influence of colonization by distinct digoxin-reducing strains of *E. lenta,* microbial interaction, and host diet (arginine). Accordingly, the variation in the abundance of these specific strains of bacteria leads to interindividual differences in the metabolism of digoxin and influences its efficacy and toxicity outcomes. This case study further emphasized the need to scrutinize the genetic and metabolic functions represented by the microbial communities to derive a more refined understanding of the role of gut microbiota in pharmacotherapy.

Indeed, more of these studies are needed to unveil the underlying mechanisms of host–gut microbiota interaction in modulating pharmacokinetics and therapeutic outcome. Such mechanistic understanding will aid the systematic characterization of drugs that are susceptible to the metabolic influence of gut microbiota and will provide insights for their therapeutic management (Jia et al., 2008; Wallace et al., 2010; Haiser and Turnbaugh, 2012, 2013; Holmes et al., 2012; LoGuidice et al., 2012; Nicholson et al., 2012; Maurice et al., 2013; Roberts et al., 2013).

Metabonomics: Mining the Metabolome to Study Host–Microbiota Interaction

Most of the established microbial influence on pharmacokinetics and therapeutic outcomes is attributed to its direct enzymatic actions on drugs. Beyond this direct action, recent studies illuminated the insidious modulatory roles of the microbial or cometabolites produced by the microbes on the host enzymatic activities. There is now an increasing awareness of the symbiotic relationship between the host and gut microbiota and their combinatorial metabolic capacities. The host metabotype is a culmination of both host systems biology and gut microbiota biology (Martin et al., 2007, 2009; Claus et al., 2008, 2011; Wikoff et al., 2009; Zheng et al., 2011; Nicholson et al., 2012; Zhao et al., 2013). Even though the gut microbiota resides in the intestines, the vena portae and lymphatic system facilitate its interaction with the host through the transportation of metabolites via the enterohepatic circulation system. Because the metabotypes are reflected within the host metabolome in biologic matrices (e.g., plasma, urine, and feces), this renders the metabolome an important source for elucidating the interaction between the host and gut microbiota.

There was previously a lack of systematic scientific techniques for elucidating the complex host–gut microbiota interaction. Fortunately, this situation has greatly improved with scientific advancement in analytical sciences, molecular biology, and bioinformatics, which, when used in combination, greatly support the endeavor of systems biology–based investigation of host–gut microbiota interaction in pharmaceutical research. Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" (Nicholson et al., 1999). Metabonomics is an attractive platform that provides access to this wealth of information embedded within the metabolome and valuable insights into the shared responsibility between the host and gut microbiota in modulating therapeutic outcome (Nicholson et al., 1999, 2012; Lindon et al., 2003, 2007; Nicholson and Wilson, 2003; Clayton et al., 2006; Han et al., 2010). The birth of metabonomics dates back to the mid-1980s (Nicholson et al., 1999). Through the use of powerful analytical instruments such as nuclear magnetic resonance spectroscopy and mass spectrometry, scientists are able to identify and quantify the small metabolites (<1500 Da) present within the metabolome (Li et al., 2008; Pasikanti et al., 2008; Wikoff et al., 2009; Yip and Chan, 2013).

The Metabonomics Workflow and Its Considerations. The typical metabonomics workflow is illustrated in Fig. 1A. Figure 1B further illustrates the various processes involved in a metabonomics study using gas chromatography time-of-flight mass spectrometry as an example. In brief, the workflow starts with the research question that defines the experimental design and the choice of biologic matrix. Because many investigations are clinically translational in nature, urine and blood samples are analyzed most frequently due to the ease of obtaining these samples (Dunn et al., 2011). The urinary and blood metabolic profiles are known to reflect variation owing to the host, drug, environment (e.g., diet, stress, and lifestyle), and gut microbiota. The samples are prepared according to the type of biologic matrix being analyzed and the instrument used for performing data acquisition (Beckonert et al., 2007; Dunn et al., 2011). For example, urine is known to contain a high concentration of urea, which will impose gas chromatographic interference. Hence, incubation with urease is performed to remove this interference during sample preparation (Pasikanti et al., 2008; Chan et al., 2011). For serum and plasma, which are rich in proteins, the proteins are first precipitated by organic solvents (Bruce et al., 2009). As for tissues, they are usually homogenized before sample extraction (Want et al., 2013). Samples that will be subjected to gas chromatography time-of-flight mass spectrometry analysis are typically subjected to chemical derivatization, such that the derivatized analytes are sufficiently volatile and thermally stable for analysis (Kaal and Janssen, 2008).

Metabolites are downstream end products of transcription and translation and are known to regulate gene expression and function as

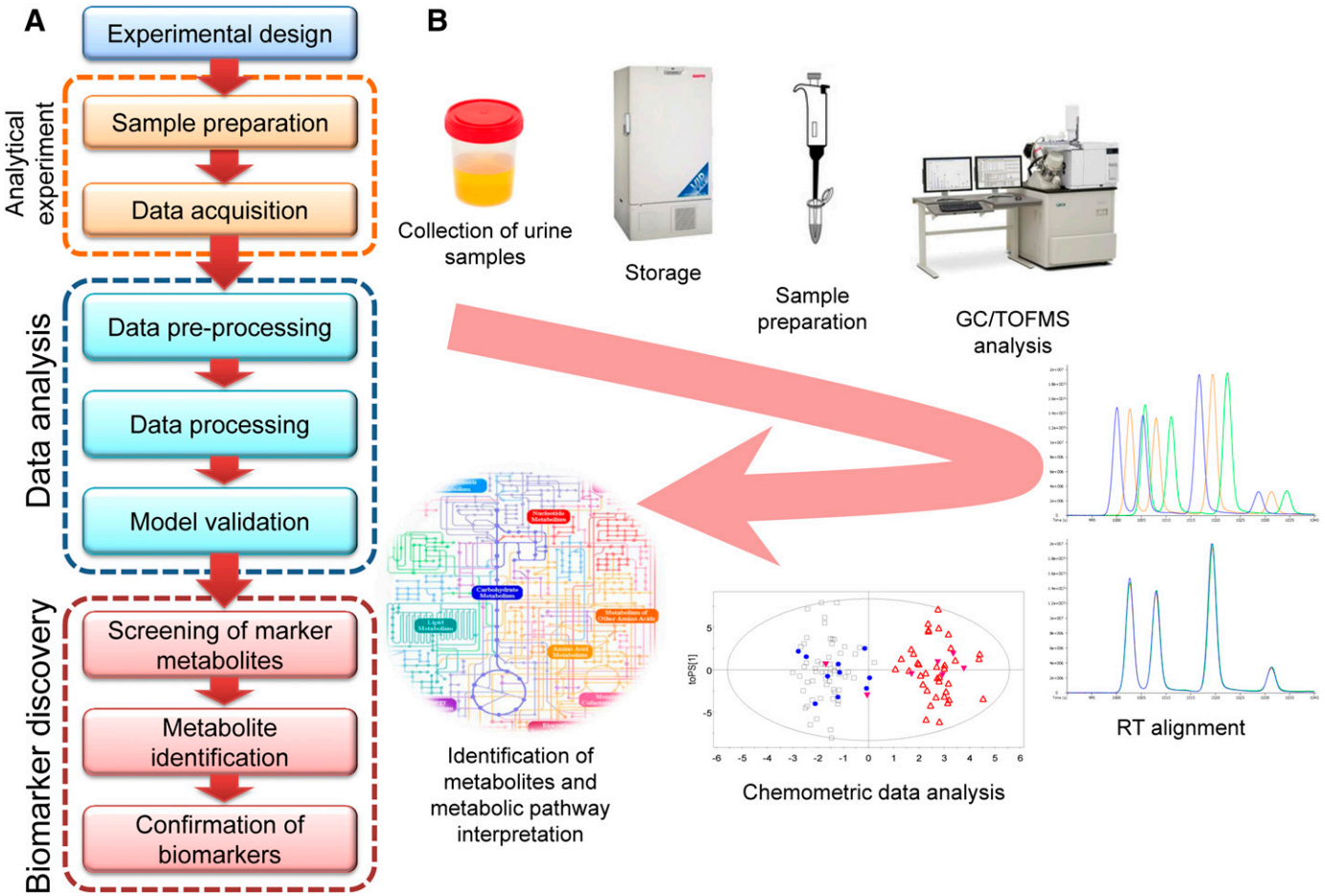

Fig. 1. (A) A typical metabonomics workflow and (B) flow diagram of various processes typically involved in GC/TOFMS-based metabonomics (Chan et al., 2011). GC/TOFMS, gas chromatography time-of-flight mass spectrometry; RT, retention time.

building blocks of more complex biologic molecules. Such metabolites include amino acids, organic acids, amines, sugars, nucleotides, fatty acids, and steroids, which span huge polarity and molecular weight ranges. Unlike DNA, RNA, and proteins, which are made up of chemically well defined building blocks, the metabolites are chemically diverse and no single analytical technique is able to measure the full complement of these metabolites. In addition, the large number of metabolites within the metabolome and the wide dynamic range of their abundances further contribute to the analytical challenges. As such, various analytical techniques such as nuclear magnetic resonance spectroscopy, gas chromatography/mass spectrometry, and liquid chromatography/mass spectrometry are often used complementarily to broaden the metabolic coverage. This complementary approach has been adopted to profile the human metabolomes in urine, blood, and cerebrospinal fluid and the findings are made available in the Human Metabolome Database (Wishart et al., 2008, 2013; Psychogios et al., 2011; Mandal et al., 2012; Bouatra et al., 2013). Metabonomics typically generates rich data, in which meaningful interpretation is made possible by systematic preprocessing of the data to workable formats for further downstream analysis using statistical tools (Wishart, 2009; Chan et al., 2011; Dunn et al., 2011; Enot et al., 2011). In applying metabonomics in pharmaceutical research, one has to be mindful of the presence of interfering chromatographic peaks derived from drugs or their metabolites, which can confound the statistical differentiation of the metabolic profiles of the drug treatment group from the control during chemometric multivariate data analysis. Manual exclusion of these peaks during the data preprocessing step is often tedious and erroneous. In such cases, our group has adopted a useful strategy in which we pool quality control samples solely from the drug-naïve controls prior to sample preparation and data acquisition (Yip and Chan, 2013). These pooled quality control samples can function as references, whereby their metabolic features are used for the alignment of the metabolic features derived from other samples during data preprocessing (Chan et al., 2011). Although this method ensures complete exclusion of all drug-related metabolic features, one needs to be mindful that selected metabolites that are highly elevated in the drug treatment group but fall below the detection limit within the control may be accidentally excluded at times.

The identities of the marker metabolites are confirmed using authentic standards and/or relevant database libraries such as the Human Metabolome Database, the National Institute of Standards and Technology (NIST) Standard Reference Database, and the Golm Metabolome Database. Metabonomics has been applied to identify marker metabolites implicated in gastroenterological diseases (Yoshida et al., 2012), central nervous disorders (Quinones and Kaddurah-Daouk, 2009), cancers (Chan et al., 2009; Mal et al., 2009; Pasikanti et al., 2010a,b; Mal et al., 2012), and kidney diseases (Weiss and Kim, 2012). Metabonomics has also been employed in preclinical toxicological screening and its usefulness has been comprehensively evaluated by the Consortium for Metabonomic Toxicology, which comprises scientists from the Imperial College London and several pharmaceutical companies (Lindon et al., 2003, 2005; Ohta et al., 2009; Aa et al., 2011; Zgoda-Pols et al., 2011).

Metabonomics Revealed Host–Gut Microbiota Modulation of Drug Disposition. More recently, metabonomics has been used to understand the interaction between the host and gut microbiota. Through the use of different experimental designs such as antibiotic-perturbed rodents (Zheng et al., 2011; Zhao et al., 2013) or germfree versus conventional rodents (Claus et al., 2008; Wikoff et al., 2009) or through gut microbiota colonization of axenic mice (Martin et al., 2007; Claus et al., 2011), metabonomic analysis of the biologic matrices (e.g., urine, plasma, fecal extracts, intestines, liver, and kidney) revealed extensive gut microbiota modulation of the host systemic metabolism. Table 2 illustrates a list of metabolites with differential abundance among hosts having different gut microbial community composition. It has been found that the gut microbial communities exert profound influence on the host's metabolism and partake in the metabolism of short-chain fatty acids, amino acids, primary and secondary bile acids, tryptophan, and carbohydrate.

The alterations of such metabolites (e.g., bile acids) as a result of combinatorial activities of the host and gut microbiota suggested broad metabolic implications on drug metabolism. In a study by Toda et al. (2009), the authors determined the effects of the intestinal microbiota on P450 expression by comparing the specific pathogen-free (SPF) and germfree mice. SPF mice are free of specific infectious microorganisms and parasites but are otherwise colonized with an undefined microbiota. They observed a higher mRNA expression of a majority of P450 enzymes in the livers of SPF mice. Using a mouse CYP3A substrate, triazolam, the authors demonstrated a higher metabolic activity of liver microsomes extracted from the SPF mice compared with germfree mice, correlating with the higher CYP3A expression in the SPF mice. Nuclear receptors such as the aryl hydrocarbon receptor, the constitutive androstane receptor, the farnesoid X receptor, and the pregnane X receptor, which modulate expression of the P450 enzymes, were also highly expressed in the livers of SPF mice compared with germfree mice. Interestingly, cholesterol-derived metabolites such as bile acids (e.g., lithocholic acid), steroid hormones, and bilirubin have been proposed to function as activators of liver nuclear receptors, and the levels of these metabolites were known to be mediated by microbial metabolism (Hylemon, 1985; Li and Chiang, 2013). As such, these studies demonstrated that the gut microbiota can modulate phase I metabolism through influencing nuclear receptor and P450 expression (Björkholm et al., 2009; Toda et al., 2009; Claus et al., 2011).

In addition, the metabolic products of the enteric bacteria are known to impose a large burden on the phase II metabolic processes of the host. Using metabonomics, Wikoff et al. (2009) revealed large effects of gut microbiota on the metabolic profiles of the mouse serum. Compared with the germfree mice, conventional mice were observed to have an exclusive presence of numerous sulfated, glycine-conjugated, and glucuronide adducts. For example, indoxyl sulfate was identified exclusively in the serum of conventional mice. This metabolite is formed as a result of the phase II sulfonation of the bacterial metabolite indole, which is derived from the enzymatic conversion of tryptophan by bacterial tryptophanase (Wikoff et al., 2009). Apart from the indole derivatives, numerous metabolites apparently resulting from phase II processing of microbial metabolites (e.g., hippuric acid, *p*-cresol sulfate, phenylacetylglycine) suggested a broad, drug-like phase II metabolic response of the host to the microbial metabolites (Wikoff et al., 2009). This metabolic burden posed by microbial metabolites is not trivial, because many drugs are rendered more polar for renal and biliary clearance by hepatic phase II metabolism. It is clear that the complex interaction between the host and gut microbiota plays a pertinent role in influencing the metabotype, which in turn underpins the variable phase II metabolic capacities among individuals with different gut microbiota composition.

Integrative Approach to Investigate Host–Gut Microbiota Interaction

As described earlier, metabonomics enables us to capture global perturbation of microbial metabolites and cometabolites within the host system. From the metabonomics findings, scientists can glean complementary insights into the host–gut microbiota interaction that accounts for variation in pharmacokinetics and therapeutic outcome. To more completely elucidate the complex nature of the interaction between the host and gut microbiota, it is necessary to adopt an integrative approach that encompasses the study of the host, microbiota, and drug (Fig. 2). This can include pharmacogenomics studies to evaluate for genetic polymorphism and in vitro assays for functional characterization of the drug target receptor, metabolic enzyme, and transporter of the host. The ability to characterize the microbial composition and to identify the implicated gut microbes and their metabolic activities plays a major part in expanding our understanding of their involvement in pharmacology. The integration of the host and gut microbiota biology using pharmacokinetics, pharmacology, toxicology, metabonomics, host genomics, and gut microbiota metagenomics data forms a holistic strategy to elucidate the complex host–gut microbiota interaction in pharmaceutical research. The following section summarizes the techniques that support the investigation of the gut microbiota.

Culture-Based and Culture-Independent Techniques. Both the culture-based technique, which has traditionally been used to study microbial metabolism in pharmaceutical research, and the more modern culture-independent approach to study the gut microbiota are valuable techniques. The initial work on the gut microbiota since the 1970s relies on culture-based techniques (Tuohy and McCartney, 2006). Through the use of selective growth media and conditions, more than 400–500 bacterial species have been identified within the human gut. The majority of gut bacteria resides in the colon and it is a major site for microbial metabolism of many endogenous and exogenous compounds (Tuohy and McCartney, 2006; Sousa et al., 2008). However, the anatomic location of the human colon prevents the direct examination of the functional activities of the microbiota. As such, in vitro determination of drug metabolism using a culture-based technique has a particular place in pharmaceutical research because it circumvents the challenges of invasive procedures, ethical drawbacks, and the costs and laborious nature associated with in vivo investigation (Sousa et al., 2008). In this regard, static batch culture is an example of an in vitro method that has been frequently employed for elucidating microbial metabolism of drugs (Coates et al., 1988; Sousa et al., 2008). Such cultures attempt to closely simulate the colonic environment by placing specific strains of bacteria, cecal, or intestinal contents (of animals) or feces (of animals or humans) into a suitable medium under careful control of factors such as anaerobic condition and pH. Drug is added to such cultures and samples are taken intermittently to quantify the amount of drug and its metabolites. It is notable that for the majority of the 40 drugs in which their pharmacokinetics is associated with microbial activity, the culture-based technique has been applied to establish the role of gut microbiota in their metabolism (Sousa et al., 2008). However, some key limitations exist with the use of culture-based techniques. Inherent to the nature of bacteria cultivation technique, this method provides an incomplete view of the phylogenetic diversity of gut microbial community because some bacteria remain uncultivable (Tuohy and McCartney, 2006). To date, less than 30% of gut bacteria species have been cultured (Fraher et al., 2012). The

TABLE 2

List of metabolites modulated in the host harboring different gut microbial community

The biologic matrices in which differential abundance of these microbial-related metabolites is reported and the respective analytical platforms (NMR, LC, or GC/MS) used for detection of these metabolites are provided to facilitate design and interpretation of metabonomic studies on host–gut microbiota interaction. Readers are encouraged to refer to the literature (e.g., Nicholson et al., 2012) for the related bacteria and biologic functions or consequences of the metabolites.

Metabolite	Biologic Matrix	Analytical Platform	Host	References
Bile acids				
Chenodeoxycholic acid	P, K, H, F	LC	Rat	Swann et al., 2011; Zheng et al., 2011
Cholic acid	P, L, K, H, F	NMR, LC	Mice, rat	Swann et al., 2011; Zheng et al., 2011; Zhao et al., 2013
Deoxycholic acid	P, L, K, F	NMR, LC	Mice, rat	Swann et al., 2011; Zhao et al., 2013
Glycochenodeoxycholate	P, K, H, L	LC	Rat	Swann et al., 2011
Glycocholic acid	P, K, H, L	LC	Rat	Swann et al., 2011
Glycodeoxycholic acid	P, K, H, L	LC	Rat	Swann et al., 2011
Hyocholic acid	K, H	LC	Rat	Swann et al., 2011
Hyodeoxycholic acid	K, H, L, F	LC	Rat	Swann et al., 2011; Zheng et al., 2011
Taurochenoxycholic acid	P, L, K, H	LC	Rat	Swann et al., 2011
Taurocholic acid	P, L, K, H, F	NMR, LC	Mice, rat	Swann et al., 2011; Zhao et al., 2013
Taurodeoxycholic acid	P, K, H	LC	Rat	Swann et al., 2011
Tauro-α-muricholic acid	K	LC	Rat	Swann et al., 2011
Tauro-β-muricholic acid	P, L, K, H, F	NMR, LC	Mice, rat	Swann et al., 2011; Zhao et al., 2013
Tauro-ω-muricholic acid	K	LC	Rat	Swann et al., 2011
Ursodeoxycholic acid	P, K, H, L	LC	Rat	Swann et al., 2011
α-Muricholic acid	L, K, H	LC	Rat	Swann et al., 2011
β-Muricholic acid	P, L, K	LC	Rat	Swann et al., 2011
ω-Muricholic acid	K, H	LC	Rat	Swann et al., 2011
Choline metabolites				
Betaine	K	NMR	Mice	Claus et al., 2008
Dimethylamine	U	NMR	Mice	Claus et al., 2008
Trimethylamine	U	NMR	Mice	Claus et al., 2008
Trimethylamine *N*-oxide	U, L	NMR	Mice	Claus et al., 2008
Phenolic, benzoyl, and phenyl derivatives				
2-(4-Hhydroxyphenyl)propionic acid	F	NMR	Mice	Zhao et al., 2013
3-(3,4-Dihydroxyphenyl)lactic acid	U	LC	Rat	Zheng et al., 2011
3-(3-Hydroxyphenyl)propanoic acid	F, U	GC	Rat	Zheng et al., 2011
3-Hydroxycinnamic acid	U	NMR	Mice	Claus et al., 2008
4-Hydroxyphenylpropionic acid	U	NMR, LC	Mice, rat	Claus et al., 2008; Zheng et al., 2011
4-Hydroxyphenylpyruvic acid	U	LC	Rat	Zheng et al., 2011
5-Phenylvaleric acid	F	LC	Rat	Zheng et al., 2011
Aminophenol	F	LC	Rat	Zheng et al., 2011
Benzoyl glucuronide	U	LC	Rat	Zheng et al., 2011
Cinnamoylglycine	P	LC	Mice	Wikoff et al., 2009
Hippuric acid	U, P	NMR, LC, GC	Mice, rat	Claus et al., 2008; Yap et al., 2008; Wikoff et al., 2009; Zheng et al., 2011
Hydroxyphenylacetylglycine	U	LC	Rat	Zheng et al., 2011
Hydroxyphenyllactic acid	F	LC	Rat	Zheng et al., 2011
m-Hydroxyphenylacetic acid	F, U	GC	Rat	Zheng et al., 2011
N-Acetyl-L-phenylalanine	U	LC	Rat	Zheng et al., 2011
p-Aminobenzoic acid	F	LC	Rat	Zheng et al., 2011
p-Cresol	U	GC	Rat	Zheng et al., 2011
p-Cresol sulfate	P	LC	Mice	Wikoff et al., 2009
Phenol	U	GC	Rat	Zheng et al., 2011
Phenyl sulfate	P	LC	Mice	Wikoff et al., 2009
Phenylacetic acid	F, U	LC	Rat	Zheng et al., 2011
Phenylacetylglycine	P, U	NMR, LC	Mice	Claus et al., 2008; Wikoff et al., 2009
Phenylalanine	F, P, L, K, D, J, I, C	NMR, LC, GC	Mice, rat	Claus et al., 2008; Yap et al., 2008; Wikoff et al., 2009; Zheng et al., 2011; Zhao et al., 2013
Phenylalanyl-hydroxyproline	F, U	LC	Rat	Zheng et al., 2011
Phenylethanolamine	F, U	LC	Rat	Zheng et al., 2011
Phenylglycine	F	LC	Rat	Zheng et al., 2011
Phenyllactic acid	F, U	LC, GC	Rat	Zheng et al., 2011
Phenylpropionylglycine	P	LC	Mice	Wikoff et al., 2009
p-Hydrocinnamic acid	F, U	GC	Rat	Zheng et al., 2011
p-Hydroxybenzaldehyde	F	GC	Rat	Zheng et al., 2011
p-Hydroxybenzoic acid	U	GC	Rat	Zheng et al., 2011
p-Hydroxyphenylacetic acid	F, U	NMR, GC	Mice, rat	Zheng et al., 2011; Zhao et al., 2013
Polyamines				
Cadaverine	F, U	GC	Rat	Zheng et al., 2011
Putrescine	F, U	NMR, GC	Mice, rat	Claus et al., 2008; Zheng et al., 2011
Spermidine	F	LC	Human	Matsumoto and Benno, 2007
Spermine	F	LC	Human	Matsumoto and Benno, 2007
Short-chain fatty acids				
Acetic acid	F, U, L, K, D, J, I, C	NMR	Mice	Claus et al., 2008; Yap et al., 2008
Butyric acid	F, U	LC, GC	Rat	Zheng et al., 2011
Hexanoic acid	F	GC	Rat	Zheng et al., 2011

(*continued*)

TABLE 2—*Continued*

Metabolite	Biologic Matrix	Analytical Platform	Host	References
Isobutyric acid	F, U	GC, LC	Rat	Zheng et al., 2011
Isovaleric acid	F, U	NMR, LC	Mice, rat	Claus et al., 2008; Zheng et al., 2011
Propionic acid	F, U	NMR, GC	Mice, rat	Yap et al., 2008; Zheng et al., 2011
Tryptophan, indole derivatives				
2-Indoleacetaldehyde	F, U	LC	Rat	Zheng et al., 2011
2-Indolecarboxylic acid	F, U	LC	Rat	Zheng et al., 2011
3-Indolepropionic acid	F	LC	Rat	Zheng et al., 2011
3-Methyldioxyindole	F	LC	Rat	Zheng et al., 2011
5-Hydroxyindoleacetic acid	U	LC	Rat	Zheng et al., 2011
5-Hydroxyindoleacetylglycine	U	LC	Rat	Zheng et al., 2011
5-Hydroxytryptophan	U	NMR	Mice	Claus et al., 2008
5-Hydroxytryptophol	F, U	LC	Rat	Zheng et al., 2011
6-Hydroxymelatonin sulfate	F, U	LC	Rat	Zheng et al., 2011
Hydroxykynurenine	U	LC	Rat	Zheng et al., 2011
Indole	U	LC	Rat	Zheng et al., 2011
Indole-3-propionate	P	LC	Mice	Wikoff et al., 2009
Indoleacetic acid	U	LC	Rat	Zheng et al., 2011
Indolelactic acid	U	LC	Rat	Zheng et al., 2011
Indoxyl	F	LC	Rat	Zheng et al., 2011
Indoxyl sulfate	P, U	LC, GC	Mice, rat	Wikoff et al., 2009; Zheng et al., 2011
Kynurenic acid	U	GC	Rat	Zheng et al., 2011
N-Acetyltryptophan	P	LC	Mice	Wikoff et al., 2009
N-Methyltryptamine	U	LC	Rat	Zheng et al., 2011
Serotonin	P	LC	Mice	Wikoff et al., 2009
Tryptamine	F, U	LC	Rat	Zheng et al., 2011
Tryptophanol	U	LC	Rat	Zheng et al., 2011
Vitamins				
Biotin	U	LC	Rat	Zheng et al., 2011
Pantothenic acid	U	LC	Rat	Zheng et al., 2011
Pyridoxal	F	LC	Rat	Zheng et al., 2011
Pyridoxine	F	LC	Rat	Zheng et al., 2011
Riboflavin	F	LC	Rat	Zheng et al., 2011
Thiamine	F	LC	Rat	Zheng et al., 2011

C, colon; D, duodenum; F, feces; GC, gas chromatography; H, heart; I, ileum; J, jejunum; K, kidney; L, liver; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; P, plasma; U, urine.

symbiotic relationship among gut microbiota species adds to the difficulties in characterizing and reproducing the appropriate growth environment for these bacteria (Miura et al., 1980; Stams, 1994; Doern, 2000). Such constraints impede culture efforts and pose challenges in creating an environment that supports the growth of all intestinal or fecal bacteria to mimic the actual colonic environment. Furthermore, culture-based techniques might be overly simplistic because they do not consider other dynamic processes that occur in intact physiologic conditions such as metabolic exchange and interaction between the host and gut microbial community and absorption of fermented products (Sousa et al., 2008). Hence, in vivo studies are conducted complementarily with in vitro studies to obtain deeper insights that are otherwise not obtainable from in vitro culture-based investigations alone. These may involve investigating microbial metabolism by comparing bile metabolites with fecal metabolites or lower gut metabolites with upper gut metabolites or comparing drug metabolism between germfree or antibiotic-treated animals with conventional animals (Meuldermans et al., 1994; Sousa et al., 2008; Yoo et al., 2014).

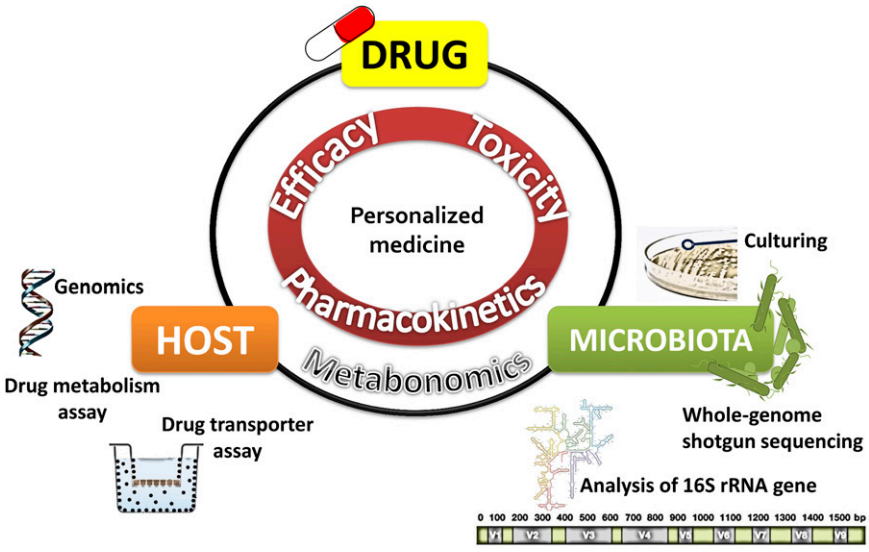

Fig. 2. Integrative approach to study host–gut microbiota interaction in pharmaceutical research.

Since the 1990s, the development of molecular biology gave rise to culture-independent techniques that revolutionized our knowledge of the gut microbiota (Fraher et al., 2012). The application of molecular tools has greatly improved our understanding of the microbial community by analyzing the structural diversity and functional activities of the microbes, even for those that cannot be cultured. The two key culture-independent approaches are based on the analysis of the 16S ribosomal RNA (rRNA) genes and the whole genome of the microbes, respectively (Blaut et al., 2002; Tuohy and McCartney, 2006; Brugère et al., 2009; Dave et al., 2012; Fraher et al., 2012; Kuczynski et al., 2012; Weinstock, 2012).

Present throughout the cytoplasm of a bacterial cell are 70S ribosomes that are made up of two subunits, the 30S and 50S subunits. The 30S subunit is known to contain the 16S rRNA. The 16S rRNA gene is universally present in bacteria and has highly conserved domains flanking nine hypervariable regions, which possess considerable sequence diversity to be used for distinguishing bacteria (Blaut et al., 2002; Chakravorty et al., 2007). As such, the 16S rRNA gene has been considered as a phylogenetic and evolutionary marker for bacteria identification (Blaut et al., 2002). The majority of the culture-independent techniques for the analysis of the gut microbiota are based on the analysis of the 16S rRNA genes. Such techniques include quantitative polymerase chain reaction (qPCR), denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, terminal restriction fragment length polymorphism, fluorescence in situ hybridization, DNA microarrays, and sequencing of 16S rRNA amplicons (Blaut et al., 2002; Tuohy and McCartney, 2006; Fraher et al., 2012). Among these techniques, qPCR has been widely employed in gut microbiota investigation because the method is fast, sensitive, and quantitative and it enables phylogenetic discrimination and targeted analysis of specific bacteria of interest through primer design (Matsuki et al., 2002; Rinttilä et al., 2004; Tuohy and McCartney, 2006; Fraher et al., 2012). However, qPCR is subjected to the inherent bias of PCR-based techniques and does not allow identification of unknown bacteria (Polz and Cavanaugh, 1998; Acinas et al., 2005; Tuohy and McCartney, 2006). On the other hand, 16S rRNA gene sequencing facilitates detection of unknown bacteria and yields information on the proportion of various microbes, which in turn allows inference on the composition of microbial communities (Fraher et al., 2012). The development of next-generation sequencing techniques (e.g., 454 Pyrosequencing, Illumina, and SOLiD) has led to a significant reduction in sequencing cost and time (Kuczynski et al., 2012), rendering these techniques to be widely adopted in research. In a study to examine the long-term effects of exogenous microbiota transplantation combined with and without an antibiotic pretreatment using a rat model, Manichanh et al. (2010) combined the application of qPCR and 16S rRNA gene sequencing to determine the degree to which the gut microbiota can be experimentally manipulated. qPCR was employed to elucidate the bacterial load present in the rat fecal samples, and a decrease in bacterial load was confirmed after the administration of antibiotics. Using 16S rRNA gene sequencing, Manichanh et al. (2010) discovered that the exogenous transplant of cecal materials from different strains of donor rats led to a change in the fecal bacterial diversity of the recipients (without antibiotic pretreatment), such that it highly resembles the donor samples and the effect persists even up to 3 months after a single inoculation. Interestingly, pretreatment with antibiotics did not facilitate the establishment of the exogenous microbiota in the recipient rats. Instead, it culminated into a greater reshaping effect, leading to a gut microbiome composition that is distant from both the donor and long-term antibiotic treatment animal. Although this result is highly counterintuitive to the authors' original hypothesis of antibiotics helping to enhance the reshaping effect of microbiota transplantation, the authors suggested that their findings should be taken into consideration during the design of future fecal microbiota transplantation studies (Manichanh et al., 2010). An integrated approach that combines 16S rRNA gene sequencing with metabonomics has also been employed in systems biology–based investigation. Using the integrated approach, Lu et al. (2014) revealed that arsenic significantly perturbed the gut microbiome composition of C57BL/6 mice. Correlation analysis further demonstrated that the abundance of selective perturbed bacteria was highly correlated with the altered gut microbiota–related metabolites (Lu et al., 2014).

The other culture-independent technique known as whole-genome shotgun sequencing involves sequencing the entire genomes of the microbes (Dave et al., 2012; Fraher et al., 2012). This method involves random fragmentation of microbial DNA, sequencing of DNA fragments, and reconstruction of overlapping sequences to form a continuous sequence (Fraher et al., 2012). Through whole-genome shotgun sequencing of the microbiome, microbial genes that encode for metabolic functions can be identified. This in turn provides insights into the potential functional activities of the microbes. In other words, whole-genome shotgun sequencing provides information on both genetic diversity and functions of the gut microbiota, whereas 16S rRNA gene sequencing provides only information on genetic diversity. However, whole-genome shotgun sequencing comes at a higher cost and is computationally more intensive. Other drawbacks include the requirement for a large amount of DNA for analysis unless genome amplification is performed and the fact that many identified genes may not currently have known function.

Conclusions

There is currently limited knowledge pertaining to the roles of gut microbiota in modulating therapeutic outcome. The diverse metabolic capabilities of these gut microbes and their variable composition in the human gut provide the impetus to scrutinize the intricate involvement of the gut microbes in accounting for interindividual variability. With the advancement in omics technologies (e.g., metabonomics and metagenomics), we envisage that the deliberate and systematic integration of these technologies will empower scientists to better investigate the roles of the gut microbiota in pharmacokinetics and pharmacotherapy. Novel drug candidates targeting the bacteria or their enzymes may be designed to modulate efficacy or toxicity (Jia et al., 2008; Holmes et al., 2012). The mitigation of irinotecan-induced gastrointestinal toxicity using β-glucuronidase inhibitors is one example (Wallace et al., 2010). Manipulation of gut microbiota may also be effected via the administration of probiotics, prebiotics, and antibiotics and via fecal transplantation (Jia et al., 2008; Holmes et al., 2012; Rohlke and Stollman, 2012). Considering the pervasive influence of gut microbes on pharmacokinetics and therapeutic outcome, careful manipulation and engineering of gut microbes may present exciting opportunities in personalized medicine.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Yip, Chan.

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