

Special Section on Drug Metabolism and the Microbiome—Commentary

Drug Metabolism by the Host and Gut Microbiota: A Partnership or Rivalry?

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ABSTRACT

The importance of the gut microbiome in determining not only overall health, but also in the metabolism of drugs and xenobiotics, is rapidly emerging. It is becoming increasingly clear that the gut microbiota can act in concert with the host cells to maintain intestinal homeostasis, cometabolize drugs and xenobiotics, and alter the expression levels of drug-metabolizing enzymes and transporters and the expression and activity levels of nuclear receptors. In this myriad of activities, the impact of the microbiota may be beneficial or detrimental to the host. Given that the interplay between the gut microbiota and host cells is likely subject to high interindividual variability, this work has tremendous implications for our ability to predict accurately a particular drug's pharmacokinetics and a given

patient population's response to drugs. In this issue of *Drug Metabolism and Disposition*, a series of articles is presented that illustrate the progress and challenges that lie ahead as we unravel the intricacies associated with drug and xenobiotic metabolism by the gut microbiota. These articles highlight the underlying mechanisms that are involved and the use of in vivo and in vitro approaches that are currently available for elucidating the role of the gut microbiota in drug and xenobiotic metabolism. These articles also shed light on exciting new avenues of research that may be pursued as we consider the role of the gut microbiota as an endocrine organ, a component of the brain-gut axis, and whether the gut microbiota is an appropriate and amenable target for new drugs.

Introduction

During the past several years, efforts focused on understanding the impact of our microbiota on human health have intensified dramatically. New technologies, coupled with approaches utilizing systems biology, have enhanced our ability to collect and interpret the copious amounts of data required for exploring the intricate relationship that exists between the host cells and their cosiderents: bacteria, viruses, and fungi. Large-scale endeavors, such as the Human Microbiome Project, have sought to characterize and compare the healthy microbiome of different anatomic sites, including the skin, oral cavity, vagina, and gut (or gastrointestinal tract) (Integrative Human Microbiome Project, 2014). Now in its second phase of implementation, this multi-institutional project is currently focused on elucidating the role of the human microbiota during pregnancy and the onset of specific diseases: inflammatory bowel diseases, type 2 diabetes, and respiratory viral infections. The microbiota of the gut is particularly intriguing because most of the total human microbiota resides in the gut, where its composition can be altered by diet, disease, the presence of pathogens, and exposure to pharmaceutical agents, in particular, antibiotics (Conlon and Bird, 2015). In fact, emerging evidence implies that the role of

the gut microbiota in metabolism is extensive, which has inspired Klaassen and Cui (2015) in this issue of *Drug Metabolism and Disposition* to propose that the gut microbiome be considered an additional drug target.

As we begin to address how the gut microbiota and host tissues interact to metabolize drugs and xenobiotics, we must first examine the physiologic roles of the gut microbiota. We then consider the multiple mechanisms by which the gut microbiota contributes to drug metabolism, including changes in host gene expression and the generation of unique metabolites.

Metabolic Function of the Gut Microbiota

The gut microbiome within an individual is established relatively early in life (Yatsunenکو et al., 2012). Infants at postnatal day 3, for example, have been found to harbor a gut microbiota population represented by an abundance of *Enterobacteriaceae* (Dogra et al., 2015). A shift in the bacterial population could be detected by 6 months of age, characterized by high levels of *Bifidobacterium* and *Collinsella* and low levels of *Enterobacteriaceae* and *Streptococcus*. Within 3 years, the phylogenetic composition of the bacterial communities found within the gut of most children closely mirrors that of the adult (Yatsunenکو et al., 2012). The colon of a healthy adult is typically highly represented by the Gram negative *Bacteroidetes* and Gram positive *Firmicutes*. Minor species that are also commonly identified include *Proteobacteria*,

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ABBREVIATIONS: AHR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; FXR, farnesoid X receptor; GLP1, glucagon-like peptide 1; GST, glutathione S transferase; IL1 β /IL4/IL5/IL6/IL10/IL12/IL13/IL17/IL21/IL22, interleukins 1 β /4/5/6/10/12/13/17/21/22; P450, cytochrome P450 enzyme; PXR, pregnane X receptor; RNA-Seq, RNA sequencing; SCFA, short-chain fatty acid; SULT, sulfotransferase; T_H, T-helper cell; UGT, UDP-glucuronosyltransferase.

Actinobacteria, and *Fusobacteria*. It is also becoming increasingly clear that individuals are host to unique microbial communities that are quite stable over time, leading some to propose that microbial “fingerprints” may soon serve as personal identifiers (Franzosa et al., 2015).

The interplay between the host cells and the microbiota exists as a two-way dialogue that hinges on metabolism as a central theme. Hence, the next challenge is elucidation of the metabolic function of specific microbial communities, once their taxonomic composition is cataloged. The gut microbiota is capable of generating a wide variety of enzymatic products that is determined, in part, by nutrient availability and the absence or presence of bacteria that competitively interact for the same enzymatic substrates (Conlon and Bird, 2015). Diets rich in carbohydrates contribute to the production of short-chain fatty acids (SCFAs), which are largely thought to be beneficial to health. High colonic butyrate concentrations enhance gut motility and limit the growth of pathogenic microorganisms. As the preferential energy source of the colonic epithelial cells, butyrate regulates the metabolic activity and proliferation of these cells. Interestingly, the generation of SCFAs varies along the length of the colon; highest levels are found within the proximal colon and lower levels within the distal colon. Commensal bacteria known to produce relatively high levels of butyrate in the gastrointestinal tract are *Faecalibacterium prausnitzii* and *Eubacterium/Roseburia* and some species of *Firmicytes*.

A healthy gut microbiota also requires sufficient amounts of protein to serve as a primary nitrogen source of colonic microbial growth. Bacterial metabolism of ingested protein can result in the formation of polyamines, hydrogen sulfide, and N-nitrosocompounds, all of which can be detrimental. At relatively low levels, polyamines participate in homeostatic activities, such as maintaining the structural integrity of membranes and nucleic acids. At high concentrations, however, catabolized polyamines contribute to oxidative stress and cellular toxicity. High levels of hydrogen sulfide produced by bacteria such as *Desulfovibrio* spp. also exert toxic actions and contribute to the loss of colonic epithelial cells, loss of intestinal barrier integrity, as well as damage to host DNA. N-nitrosocompounds, which can be produced by bacteria such as *Proteobacteria*, are also mutagenic and capable of damaging the mucosal layer. With respect to dietary fats, excess consumption of saturated fats leads to an increase in the growth of *Clostridium* clusters XI/XIVa and sulfate- or sulfite-reducing bacteria (Shen et al., 2014), which is thought to ultimately result in an increase in the formation of proinflammatory and genotoxic secondary bile acids, such as deoxycholate.

Contribution of the Gut Microbiota to Intestinal Immune Homeostasis

The gastrointestinal tract is a site of exposure to both deleterious pathogens and commensal bacteria (Danese, 2011; Zhang and Li, 2014; Bates and Diehl, 2014). The default state of the gut is one of hyporesponsiveness where the host response to pathogens is attenuated and the presence of commensal bacteria and food antigens is tolerated. Within the colon, the commensal bacteria colonize within the outer loose layer of mucus (Johansson et al., 2011) and contribute to intestinal homeostasis by activating resident immune cells (macrophages, neutrophils, innate lymphoid cells, B cells, and T cells) such that they produce antimicrobial factors (Maranduba et al., 2015).

The adaptive immune response within the gut is particularly sensitive to the presence of microorganisms. The differentiation of naïve CD4 T cells is a highly regulated process involving the formation of four subsets, T-helper (T_H)1, T_H 2, T_H 17, and T_{reg} cells, each of which is characterized by their secretion of predominant cytokines. T_H 1 cells are best known for their production of IFN γ , as well as TNF α . T_H 2 cells secrete primarily interleukin (IL)4, IL5, and

IL13, whereas T_H 17 cells produce IL17, IL21, and IL22. Finally, T_{reg} cells secrete the anti-inflammatory cytokine IL10. The T_H 17-mediated response plays a critical role in balancing the anti-inflammatory versus proinflammatory responses as its primary function is to restrain the T_{reg} cells from suppressing the T_H 1 response. The presence of specific microorganisms can determine which T-helper subset predominates within the gastrointestinal tract and thus determine whether the milieu of the gut is predominately proinflammatory versus anti-inflammatory (McDermott and Huffnagle, 2014; Maranduba et al., 2015). For example, polysaccharide A, produced by bacteria such as *Bacteroides fragilis*, activates the toll-like receptor 2 (TLR2), which enhances formation of the T_{reg} cells and thus enhances secretion of the anti-inflammatory cytokine, IL10. The presence of segmented filamentous bacteria, however, generates a proinflammatory (yet protective) response via increased formation of T_H 17 cells. Finally, an overabundance of pathogenic bacteria induces secretion of proinflammatory cytokines (i.e., IL1 β , IL6, IL12, and IL13) by the intestinal epithelial cells, activated dendritic cells, and macrophages. The ability of commensal, beneficial bacteria to enhance formation of T_{reg} cells is due largely to the metabolites that they produce, SCFAs.

Additional microbial metabolites that alter T-cell differentiation are those derived from tryptophan. The generation of a tryptophan metabolite, indole-3-aldehyde, by *Lactobacilli* is of particular note as it has been found to enhance production of IL22, via the aryl hydrocarbon receptor (AHR), and thereby offer protection against colonic inflammation (Zelante et al., 2013). In the gut, secretion of IL22 by innate lymphoid and T_H 17 cells can promote proliferation of the gut epithelial cells (Kumar et al., 2013). The AHR is a member of the basic helix-loop-helix Per-Arnt-Sim family (Kohle and Bock, 2009; Murray et al., 2014) that has been historically of interest because of its ability to regulate the expression levels of drug metabolizing enzymes and transporters. Genes typically upregulated by the AHR are cytochromes CYP1A1 and CYP1B1 (phase 1); GSTA1, GSTA2, and UDP-glucuronosyltransferase UGT1A1 (phase 2); and multidrug resistance associated protein MRP3/ABCC3 (phase 3). The ability of the AHR also to regulate immune function and intestinal homeostasis in a manner that appears to involve microbiota-generated metabolites is currently of high interest. With this in mind, Hubbard et al. (2015) focused on our emerging understanding of the metabolic formation of endogenous AHR ligands from tryptophan and indole by both the host and gut microbiota. In addition, they speculated on how the absence or presence of these metabolites may impact gut homeostasis, barrier function, and the gut inflammatory response via their AHR modulating activities. The extent to which the tryptophan metabolites by the gut microbes activate or inhibit the AHR is addressed by the work performed by Cheng et al. (2015). Here, evidence is provided that these microbial tryptophan metabolites exhibit varying properties with respect to their ability either to activate or to inhibit the AHR and are shown to act as SAhRMs or selective AHR modulators in that they act in a cell-context and gene-specific manner. Of particular interest are the tryptophan metabolites tryptamine and indole-3 acetate.

Role of the Gut Microbiota in Bile Acid Metabolism

The gut microbiota plays an extensive role in bile acid metabolism and in this manner contributes to the health of the host via its impact on the absorption of lipids and lipid-soluble vitamins and maintenance of intestinal barrier function (Li and Jia, 2013; Swanson et al., 2013; Klaassen and Cui, 2015). Whereas the liver is the major source of primary bile acid synthesis, the intestinal bacteria are largely responsible for the production of secondary bile acids. The physiologic actions of bile acids are thought to arise primarily from their activation or

inhibition of nuclear receptors, in particular, the FXR as well as the membrane G-protein-coupled receptor, TGR5. The FXR is involved in feedback inhibition of bile acid metabolism and modulation of lipid and glucose metabolism. Agonist activation of FXR leads to the repression of CYP7A1 and CYP8B1 expression and upregulation in the expression levels of CYP3A4, CYP3A11, SULT2A1, UGT2B4, and transporters such as ABCB11 and ABCB4. Bile acids such as chenodeoxycholic and cholic acid are well characterized FXR agonists, whereas tauro-conjugated β - and α -muricholic acids (i.e., T β MCA) have recently been identified as FXR antagonists (Sayin et al., 2013). Agonist activation of TGR5 by secondary bile acids (generated by the intestinal microbiota), such as lithocholic acid and taurolithocholic acid, also plays a key role in the regulation of glucose homeostasis and energy expenditure (Swanson et al., 2013). An important TGR5 target gene is GLP1 (glucagon-like peptide 1), a gut hormone that induces glucose-dependent stimulation of insulin, stimulates the proliferation and differentiation of insulin secretion, and delays carbohydrate absorption. Previous studies have shown that in mice, colonization with gut bacteria can directly regulate signaling of the FXR in the gut and, in this manner, modify bile acid metabolism and potentially lipid and glucose homeostasis (Sayin et al., 2013). In this issue, Selwyn et al. (2015a) provide evidence that the gut microbiome may contribute to the generation of bile acids that are capable of acting as agonists of TGR5 in concentrations sufficient for increasing ileac secretion of the TGR5 target, GLP-1.

Gut Microbiota Is an Endocrine Organ and “Second Brain”?

It has recently been proposed that the gut microbiota be considered an endocrine organ as it is capable of generating a number of chemical substances that directly interact with and activate specific receptors (Clarke et al., 2014). Further, the substances that are produced by the gut microbiota can be effective at relatively low concentrations and impact distant organs, such as the brain. In addition to SCFAs, tryptophan metabolites, and bile acids mentioned already, the gut microbiota can produce a number of neurotransmitters, including serotonin, dopamine, and noradrenaline, as well as tryptophan that is converted to 5-hydroxytryptamine. Gut microbial generation of neurotransmitters—in particular, serotonin—has spurred other researchers to refer to the gut microbiota as a “second brain” (Ridaura and Belkaid, 2015). Interestingly, a diverse number of metabolites generated by the gut microbiota can impact serotonin production and, in this manner, participate in the gut-brain axis to form the microbiota-gut-brain axis. As described in this issue (Rosenfeld, 2015), however, the gut microbiota also generates metabolites, such as 4-ethylphenylsulfate, SCFA, and ammonia, which may exert adverse neurobehavioral effects. With this in mind, Rosenfeld examines the interplay that exists within the microbiota-gut-brain axis and queries whether gut dysbiosis and aberrant gut metabolism may lead to autism-like disturbances.

Impact of the Gut Microbiota on the Drug-Metabolizing Enzymes, Transporters, and their Regulators

The expression levels of drug-metabolizing enzymes and transporters are regulated by several nuclear receptors, in particular, the CAR and PXR and, as previously mentioned, the FXR and AHR (Kohle and Bock, 2009; Gadaleta et al., 2015). Each nuclear receptor is capable of upregulating a coordinate set of phases 1, 2, and 3 enzymes and transporters that may be distinct or may overlap with that of other nuclear receptors. CAR, PXR, and FXR are members of the steroid receptor superfamily that regulate their cognate genes via formation of a DNA-binding heterodimer with retinoid X receptor

(Kohle and Bock, 2009). Drug-metabolizing enzymes and transporters that are upregulated by CAR include cytochromes CYP2B6 and CYP2C9 (phase 1), UGTB1, SULT1E1 (phase 2), and organic anionic transport protein OATP1B3 (phase 3). With respect to phase 1 and phase 2 metabolizing enzymes, PXR regulates CYP3A, CYP2B, and CYP2C and GSTA1, UGT1A3, and UGT 1A6, respectively. PXR and CAR regulate overlapping sets of genes involved in phase 1, phase 2, and phase 3 metabolism.

To elucidate the impact of the microbiota on hepatic expression levels of drug-metabolizing enzymes, an analysis of gene expression in germ-free versus conventionally raised female C3H/Orl mice was performed (Claus et al., 2011). Here, the hepatic levels of Cyp2c29, Cyp3a11, and Cyp8b1 were significantly lower in the germ-free mice. After 20 days of bacterial colonization and adaptation, however, Cyp2c29, Cyp3a11 and Cyp8b1 levels were no longer reduced, and increases in Cyp2d9 and Cyp2e1 were observed in the germ-free mice compared with the conventionally raised mice. With respect to nuclear receptor expression, the germ-free mice harbored higher mRNA levels of CAR, FXR, and PXR, whereas AHR, PPAR α , and retinoid X receptor α mRNA levels were unchanged after 20 days of microbial colonization of the gut.

In this issue, Selwyn et al. (2015b) extend these findings using an unbiased method of quantitating and comparing mRNA abundance, RNA-Seq, to identify changes in the expression levels of hepatic drug-metabolizing enzymes in germ-free versus conventionally raised C57BL male mice. In addition to providing a more extensive analysis of the impact of the gut microbiota on hepatic expression of drug-metabolizing enzyme, the work (Selwyn et al., 2015b) reports findings that contradict that of previous reports (Bjorkholm et al., 2009; Toda et al., 2009), which may be indicative of differences in strains, housing, environments or diet.

Impact of Drugs and Xenobiotics on the Composition and Function of Gut Microorganisms

Several classes of drugs and xenobiotics have been reported to alter the composition of the gut microbiome in a manner that is thought to be detrimental to health (Maurice et al., 2013; Carmody and Turnbaugh, 2014). For example, patient use of proton pump inhibitors has been associated with *Clostridium difficile* infections (Kwok et al., 2012). A recent analysis investigating the impact of a variety of drugs, including antibiotics, digoxin, phenacetin, and sulfasalazine, indicated that antibiotics had the greatest impact on the functional activity of the gut microbiome (Maurice et al., 2013). The extent to which antibiotic treatment modulates the metabolism of orally administered drugs is further scrutinized in this issue (Kim, 2015). In addition to drugs, a number of xenobiotics can alter the gut microbiota. Of these, perhaps the best characterized is arsenic, which has been shown in a mouse model to decrease significantly the abundance of *Firmicutes* (producers of butyrate) and alter the composition of indole and glucuronide metabolites (Lu et al., 2014).

Impact of the Microbiota on the Metabolism and Bioavailability of Phytochemicals

An important function of host-microbial cometabolism is its conversion of dietary plant substances into bioactive molecules (Carmody and Turnbaugh, 2014). This role has attained increasing importance as our use of traditional medicines and herbal supplements becomes more popular. Dietary plant substances that are the most susceptible to microbial metabolism in the human colon are the phytochemicals (phenolics and flavonoids). The impact of the gut microbiota on

phytochemicals includes metabolic conversions involving esterases, glycosidases, demethylations, dehydroxylations, and decarboxylations (Laparra and Sanz, 2010).

Curcumin is among the best studied naturally occurring phenolics due to its medicinal properties that are linked to its anti-inflammatory and anti-oxidant activities (Wu et al., 2014). The pharmacologic activity of curcumin is thought to be due to the formation of its metabolite tetrahydrocurcumin by the gut microbiota. Analyses of microorganisms isolated from human feces revealed that *Escherichia coli* exhibited among the highest curcumin-metabolizing activities (Hassaninasab et al., 2011). The responsible enzyme was identified as CurA, NADPH-dependent curcumin/dihydrocurcumin reductase, which bears similarities to members of the medium-chain dehydrogenase/reductase superfamily. In addition to mediating the conversion of curcumin to tetrahydrocurcumin, CurA also showed an ability to metabolize another phenolic, resveratrol.

Flavonoids are typically absorbed by the small intestine and colon as glycosides (Del Rio et al., 2013). Within the enterocytes, they are converted to sulfates, glucuronides, and methylated metabolites. Upon entering the liver, they are subject to further phase I metabolism. A considerable amount of flavonoid metabolites is excreted in the urine. The bioavailability of flavonoids is quite low, ranging from 2.5% to 18.5% of the consumed flavonoid, and is dependent in large part on the extent to which they are metabolized by enzymes expressed by the host and microbiota; however, the extent to which flavonoids are excreted in the urine, metabolized by the colonic microflora to circulate in the plasma, or sequestered within a given tissue is dependent on the flavonoid subclass and the complexity of its structure. Flavonoids are present in relatively high concentrations in traditional medicines, where they are often thought to be the most active ingredients. An example is the Chinese medicine, *Epimedium*, used to treat osteoporosis (Li et al., 2015). *Epimedium* is produced from the dried leaves of *Epimedium* L and contains 141 different flavonoids, of which the most abundant and bioactive is icariin. As previously stated, the gut microbiota plays an important role in flavonoid metabolism, and disease conditions often alter the composition of the gut microbiome. Hence, Zhou et al. (2015), in this issue, questioned whether conditions of osteoporosis may modulate the metabolism of the major flavonoids present in *Epimedium* in a manner that may ultimately affect its bioavailability and efficacy.

Flavonoids are also important constituents of *Astragalus radix*, a traditional Chinese herbal medicine used to treat a wide variety of disease states for its anti-inflammatory and other properties (Fu et al., 2014). Ruan et al. (2015) in this issue examine the extent to which the rat gut microbiota alter glucuronidation and some pharmacologic properties of the most abundant flavonoid present in *Astragalus radix*, calycosin-7-O- β -D-glucoside. In addition, they provide evidence that calycosin-7-O- β -D-glucoside may alter the composition of the gut microbiome in part via promoting the growth of beneficial organisms such as *Lactobacillus* and *Bifidobacterium*.

Impact of the Gut Microbiome on the Metabolism and Pharmacokinetics of Drugs and Xenobiotics

The gut microbiome uses a number of diverse mechanisms to alter the disposition, efficacy and toxicity of drugs and xenobiotics as follows (Carmody and Turnbaugh, 2014; Klaassen and Cui, 2015): 1) The gut microbiota may express enzymes that either metabolically activate or inactivate drugs. For example, sulfalazine used to treat gut inflammation is converted to its pharmacologically active form, 5-amino 5-salicylic acid by microbial enzymes. In contrast, digoxin is inactivated by a "cardiac glycoside" expressed by *Eggerthella*

lenta. 2) The drug may be sequestered by direct binding to the bacterial organism. An example here is the sequestration of L-DOPA by *Helicobacter pylori*. 3) The drug may be metabolically reactivated by microbially expressed enzymes. A good example of this mechanism is provided by the chemotherapeutic drug irinotecan (also called CPT-11) (Wallace et al., 2010). In the liver, irinotecan is metabolically inactivated via glucuronidation. Within the intestines, however, it is then metabolically reactivated by bacterially expressed β -glucuronidase resulting in diarrhea. 4) The microbiota may generate metabolites that act as metabolic intermediates. For example, the toxicity of melamine is due in large part to the microbial formation of its metabolite cyanuric acid (Carmody and Turnbaugh, 2014). 5) Finally, the microbial (p-cresol) and host metabolites of a given drug (acetaminophen) may directly compete for a host enzyme (SULT1A1).

Metabolic reactions mediated by the microbiota that are known to significantly impact the biologic activity of drugs and xenobiotics involve reduction, hydrolysis, dihydroxylation, acetylation, deacetylation, proteolysis, deconjugation, and deglycosylation processes (Sousa et al., 2008). Although more than 30 commonly prescribed drugs have been shown to be metabolically altered by the gut microbiota, an increasing body of literature continues to extend the number of drugs that are subject to bacterial metabolism in the gut and other tissues. The studies described as follows provide a tantalizing glimpse into the emerging role of the gut microbiota in drug metabolism and pharmacokinetics.

Bacterial nitroreduction reactions are of considerable interest because they can significantly impact the pharmacologic activity of nitroaromatic drugs such as chloramphenicol (Roldan et al., 2008), 2-chloro-5-nitro-N-phenylbenzamide (GW9662), (Kapetanovic et al., 2012), nitrobenzodiazepine (LinWu et al., 2012), and CB1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] (Prosser et al., 2010). Chloramphenicol, an antibiotic, was one of the first drugs discovered to be a substrate of bacterial nitroreductases (Roldan et al., 2008). GW9662 is an antagonist of peroxisome proliferator activated receptor γ and a potential chemopreventive agent. The predominant metabolite of GW996 in the plasma has been identified as an amine metabolite, and its nitroreduction by bacterial nitroreductases can significantly alter its mutagenicity (Kapetanovic et al., 2012). Study of the nitroreduction of nitrobenzodiazepine, an addictive sedative used to treat anxiety and sleep disorders, has led to further characterization of bacterial nitroreductases involved in its metabolism (LinWu et al., 2012). The involved nitroreductase has been identified as NfsB, which is expressed by *E. coli*. Since nitroreduction leads to the inactivation of nitrobenzodiazepine, it is proposed that NfsB may be useful for developing antiaddictive agents. The anticancer drug CB1954, a dinitrobenzamide prodrug, was developed to specifically target cancer cells via the delivery of the NfsB transgene (Prosser et al., 2010). Additional enzymes expressed by *E. coli* species that are capable of azo and nitro reduction, at least under aerobic conditions include AzoR and NfsA (Mercier et al., 2013). Nitroreductases are also expressed by other microorganisms, including species of *Bacillus*, *Mycobacterium*, *Bacillus*, *Enterobacter*, and *Staphylococcus* (Roldan et al., 2008). Although nitroreductases are known to play a role in the development of antibiotic resistance, their role in metabolizing currently prescribed drugs is yet to be determined.

Bacterially mediated N-oxide reduction lies at the core of an interesting interplay between the host and microbial enzymes in the metabolism of BILR355, an inhibitor of the human immunodeficiency virus (Li et al., 2012). BILR355 is extensively metabolized by CYP3A; however, study of the concomitant administration of ritonavir with BILR355 uncovered

a unique metabolic role of gut bacteria and aldehyde oxidase. Here, the biotransformation was found to involve a two-step process. In the first step, the reduced form of the N-oxide is generated by the gut bacteria. In the second step, the bacterially derived metabolite is subject to further metabolism by the host enzymes, CYP3A or aldehyde oxidase. In the presence of ritonavir, however, CYP3A activity is compromised, and the bacterial/aldehyde oxidase mediated reactions predominate.

Interplay between host cytochrome P450 and gut bacterial enzymes is also involved in the metabolism of fostamatinib, a tyrosine kinase inhibitor (Sweeny et al., 2010). Fostamatinib is a prodrug that, upon cleavage by alkaline phosphatases, is oxidatively metabolized by CYP3A4. Phase 2 metabolites include glucuronide and sulfate conjugates. In addition, in feces, a metabolite has been identified that is thought to be formed via *O*-demethylation and dihydroxylation by the anaerobic gut bacteria.

Use of an in vitro colon model coupled with metabolomics has revealed that simvastatin can be extensively metabolized by the colon microbiota (Aura et al., 2011). Simvastatin is a lactone prodrug that is designed to inhibit 3-hydroxy-3-methylglutaryl coenzyme A and reduce cholesterol levels. In the liver, simvastatin is hydroxylated and subjected to β -oxidation, glutathione conjugation, and glucuronidation. Metabolites formed by the colon bacteria are thought to arise from demethylation, carbon-carbon bond cleavage, α or β oxidation, dihydroxylation, and cyclization of simvastatin.

Other drugs determined to be significantly metabolized by colonic bacteria using in vitro cultures include prednisolone, a glucocorticoid agonist and anti-inflammatory agent (Yadav et al., 2013), and ranitidine, an H₂ antagonist (Basit and Lacey, 2001). The potential impact of the microbiota on drug pharmacokinetics has also been examined using more indirect approaches. For example, the administration of a live probiotic (*E. coli* Nissle 1917) to rats increased the bioavailability of amidarone, an antiarrhythmic agent (Matuskova et al., 2014); however, it is yet to be determined whether these changes in the pharmacokinetics of amidarone are due to alterations in drug transport or bacterial metabolism. A pilot study performed in human patients also indicates that the microbiome may alter the pharmacokinetics of tacrolimus, a calcineurin inhibitor (Lee et al., 2015). Since tacrolimus exhibits a narrow therapeutic index, its dosage is often titrated and carefully monitored to ensure that optimal therapeutic drug levels are achieved. Analyses of the fecal microbiota of 19 patients involved in this study indicate that those requiring higher doses of tacrolimus also harbor an abundance of fecal *Faecalibacterium prausnitzii*. It remains to be determined whether an abundance of commensal bacteria, like *Faecalibacterium prausnitzii*, which are often associated with a “healthy gut” (Scott et al., 2015) corresponds to an “optimal” drug-metabolizing capacity in the gut.

Xenobiotics that have been shown to be subjected to microbial metabolism in the gut include arsenic and polyaromatic hydrocarbons. Recent findings using a simulator of the human gut microbiota indicate that the colon microbiota can participate in extensive metabolism of arsenic (Rubin et al., 2014). Of key importance are the sulfate-reducing bacteria, which via their production of H₂S convert monomethylarsonic acid to monomethyl monothioarsonate, a more toxic form of arsenic. The involved bacteria are thought to be primarily *Desulfovibrio desulfuricans*.

Polyaromatic hydrocarbons are toxic human carcinogens that are formed primarily from the incomplete combustion of fossil fuels (Ball and Truskewycz, 2013). Their metabolism involves oxidation reactions by cytochrome P450s, followed by phase 2 conjugation with typically glucuronic acid, glutathione, or sulfate. Recent results obtained using a simulator of the human microbiota indicate that the microbiota obtained from the human colon, but not the stomach or small intestine, is capable of biotransforming the polyaromatic

compounds naphthalene, phenanthrene, pyrene, and benzo[a]pyrene (Van de Wiele et al., 2005). Interestingly, the reactions appeared to involve the formation of hydroxyl metabolites, which, unlike the parent compounds, exhibit estrogenic activities. Additional work has been performed using microorganisms isolated and cultured from human skin (Sowada et al., 2014). Here, the microorganism most commonly identified that was capable of metabolizing benzo[a]pyrene was *Micrococcus luteus*, and the most likely involved enzyme was identified as a DszA/NtaA-like oxygenase.

The toxicity of hydrazine has also been reportedly altered by the presence of the gut microbiota (Swann et al., 2009). Hydrazine and its derivatives are used as a rocket propellant and in a number of industrial processes and the synthesis of agricultural chemicals (Choudhary and Hansen, 1998). Adverse effects associated with human exposures to hydrazines include hepatotoxicity, reproductive and neurologic effects, and cancer. Hydrazines are subject to acetylation by N-acetyl transferase and oxidation by P450s 1A1, 1A2, 2B1, and 2E2. Whereas germ-free rats exhibited greater toxicity in response to a single orally administered dose of hydrazine (60 mg/kg) compared with their conventionally raised counterparts, the toxic effects were thought to arise from enhanced neurotoxicity and elevated levels of 2-aminoadipate rather than differences in hydrazine metabolism (Swann et al., 2009).

Accurate predictions of drug metabolism within a specific patient population will most likely require a measure of the extent to which the gut contributes to a given drug or xenobiotic's metabolic activation or inactivation status. This topic is undertaken by McCabe et al. (2015) in this issue; they used a combination of approaches to elucidate the impact of the gut bacteria on the metabolism of deleobuvir, a non-nucleoside polymerase inhibitor used to treat hepatitis C infections. This study also provides insights into the limitations and challenges associated with the use of in vivo and in vitro approaches to be used for studying the cometabolism of drugs in the host and gut microbiota.

Our quest to deliver a personalized approach to medicine necessitates a thorough understanding of the myriad of factors that contribute to interindividual differences in drug responses. Although we have made tremendous strides in predicting the impact of genetic polymorphisms in drug-metabolizing enzymes and transporters, the development of the tools and approaches necessary for anticipating how the microbiome contributes to these variations is in its initial stages. In this issue, Yip and Chan (2015) review host-gut microbial interactions that influence the pharmacokinetics and therapeutic effects of a number of drugs. They then discuss the use of metabolomics and both culture-based and culture independent approaches that can be used to determine the extent to which the gut microbiota contributes to interindividual responses to drugs.

Conclusions

Like the host enterocytes and hepatocytes, gut microorganisms actively participate in determining the bioavailability, efficacy, and side effects of orally administered drugs, xenobiotics, and dietary substances. As we continue to expand our understanding of how the gut microbiota contributes to the metabolism of drugs and xenobiotics, we must develop more advanced experimental approaches to define more completely its overall impact on patient response, the factors that contribute to interindividual differences, and the mechanisms that underlie the host-microbiome interplay. These advances will not only allow us to improve our ability to predict an individual's response to specific drugs and xenobiotics, but they will also provide new opportunities for exploiting the host-microbiome relationship to develop either more effective or safer therapies.

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