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

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Running Title: Drug metabolism and the gut microbiome

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Abbreviations

AHR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; CYP, cytochrome P450 enzyme; FXR, farnesoid X receptor; GST, glutathione S transferase; IL1β/IL4/IL5/IL6/IL10/IL12/IL13/IL17/IL21/IL22, interleukins 1β/4/5/6/10/12/13/17/21/22; MRP, multidrug resistance associated protein; OAT, organic anionic transport protein; PXR, pregnane X receptor; RXR, retinoid X receptor; RNA-Seq, RNA sequencing; SCFA, short chain fatty acids; SULT, sulfotransferases; TLR, toll-like receptor; UGT, UDP-glucuronosyltransferase

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Abstract

The importance of the gut microbiome in not only determining 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, co-metabolize drugs and xenobiotics and alter the expression levels of drug metabolizing enzymes, 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 inter-individual variability, this work has tremendous implications for our ability to accurately predict a particular drug's pharmacokinetics and a given patients 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. The articles highlight the underlying involved mechanisms and the use of in vivo and in vitro approaches that are currently available for their use in elucidating the role of the gut microbiota in drug/xenobiotic metabolism. They 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.

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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 system 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 co-residents; bacteria, viruses and fungi. Large scale endeavors, such as the Human Microbiome Project, have sought to characterize and compare the healthy microbiome of different anatomical sites including the skin, oral cavity, vagina and gut (or gastrointestinal tract)(Integrative, 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 and type 2 diabetes and respiratory viral infections. The microbiota of the gut is particularly intriguing as the vast majority 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, the emerging evidence implies that the role of the gut microbiota in metabolism is extensive which has inspired Klaassen and Cui (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 physiological roles of the gut microbiota. We will then consider the multiple mechanisms by which the gut microbiota contributes to drug metabolism which includes changes in host gene expression and the generation of unique metabolites.

The metabolic function of the gut microbiota

The gut microbiome within an individual is established relatively early in life (Yatsunenko et al., 2012). Infants at post-natal day 3, for example, have been found to harbor a gut microbiota population that was represented by an abundance of *Enterobacteriaceae* (Dogra et al., 2015*)*. A shift in the bacterial population could be detected by 6 months of age which

was characterized by high levels of *Bifidobacterium* and *Collinsella* and low levels of *Enterobacteriacea* and *Streptococcus*. Within 3 years of age, the phylogenetic composition of the bacterial communities found within the gut of the majority of children closely mirrors that of the adult (Yatsunenko et al., 2012). The colon of a healthy adult is typically highly represented by the gram negative *Bacteroidetes* and gram positive *Firmicytes*. Minor species that are also commonly identified include *Proteobacteria*, *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 dialog that hinges on metabolism as a central theme. Hence, the next challenge to be surmounted is the 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 (SCFA) 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 SCFA varies along the length of the colon with the highest levels found within the proximal colon and lower levels within the distal colon. Commensal bacteria that are 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 sulphide, and N-nitrosocompounds, all of which can be detrimental. When present at relatively low levels, polyamines participate in

homeostatic activities such as maintaining the structural integrity of membranes and nucleic acids. When present at high concentrations, however, catabolized polyamines contribute to oxidative stress and cellular toxicity. High levels of hydrogen sulphide 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 lead to an increase in the growth of *Clostridium* clusters XI/XIVa and sulfate/sulfite-reducing bacteria (Shen et al., 2014). This is thought to ultimately result in an increase in the formation of pro-inflammatory 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 (Bates and Diehl, 2014; Danese, 2011; Zhang and Li, 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 mucus layer (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, the T_H1, T_H2, T_H17, and T_{reg} cells, each of which is characterized by their secretion of predominate cytokines. T_H1 cells are best known for their production of IFNγ as well as TNFα. T_H2 cells secrete primarily IL4, IL5, and IL13, whereas T_H17 cells produce IL17, IL21, and IL22. Finally, T_{reg} cells secrete the anti-inflammatory cytokine IL10. The T_H17-mediated response plays a critical role in balancing the anti-inflammatory versus pro-inflammatory responses as its primary function is to restrain the T_{reg} cells from suppressing the

T_H1 response. The presence of specific microorganism can determine which T-helper subset predominates within the gastrointestinal tract and thus determine whether the milieu of the gut is predominately pro- versus anti-inflammatory (Maranduba et al., 2015; McDermott and Huffnagle, 2014). 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 pro-inflammatory (yet protective) response via increased formation of T_H17 cells. Finally, an overabundance of pathogenic bacteria induces secretion of pro-inflammatory cytokines (i.e., IL1β, 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 largely due to the metabolites that they produce, SCFA.

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_H17 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 CYP1A1 and CYP1B1 (phase I), GSTA1 and GSTA2 and UGT1A1 (phase II), andMRP3/ABCC3 (phase III). The ability of the AHR to also 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, T.D. et al., (Hubbard et al., 2015) focus 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, the authors speculate 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 to either activate or 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 (Klaassen and Cui, 2015; Li and Jia, 2013; Swanson et al., 2013). While 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 physiological actions of bile acids are thought to arise primarily from their activation and/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 beta and alpha-muricholic acids (i.e., TβMCA) 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

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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.

The 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 SCFA, tryptophan metabolites and bile acids mentioned above, the gut microbiota can produce a number of neurotransmitters including serotonin, dopamine and noradrenaline as well as tryptophan that is converted to 5-HT. Gut microbial generation of neurotransmitters, in particular, serotonin, has spurred other authors 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. However, as described in this issue (Rosenfled, 2015), 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 (Gadaleta et al., 2015; Kohle and Bock, 2009). Each nuclear receptor is capable of upregulating a coordinate set of phases I, II, and III 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 RXR (Kohle and Bock, 2009). Drug metabolizing enzymes and transporters that are upregulated by CAR include CYP 2B6 and CYP2C9 (phase I), UGT B1, SULT 1E1 (phase II), and OATP1B3 (phase III). With respect to phase I and phase II metabolizing enzymes, PXR regulates CYP3A, CYP2B and CYP2C and GSTA1, UGT1A3 and UGT 1A6, respecitvely. PXR and CAR regulate overlapping sets of genes involved in phase I, phase II, and phase II metabolism.

To elucidate the impact of the microbiota on hepatic expression levels of drug metabolizing enzymes, an analyses 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. However, after 20 days of bacterial colonization and adaption, Cyp2c29, Cyp3a11 and Cyp8b1 levels were no longer reduced and increases in Cyp2d9 and Cyp2e1 were observed in the germ-free as compared to the conventionally raised mice. With respect to nuclear receptor expression, the germ-free mice harbored higher mRNA levels of CAR, FXR and PXR, while AHR, PPARα and RXRα mRNA levels were unchanged after 20 days of microbial colonization of the gut.

In this issue, (Selwyn et al., 2015b) Selwyn et. al., 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 analyses 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.

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The 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 (Carmody and Turnbaugh, 2014; Maurice et al., 2013). For example, patient use of proton pump inhibitors has been associated with *Clostridium difficule* infections (Kwok et al., 2012). A recent analyses investigating the impact of a variety of drugs, including antiobiotics, 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 significantly decrease the abundance of *Firmicytes* (producers of butyrate) and alter the composition of indole and glucuronide metabolites (Lu et al., 2014).

The impact of the microbiota on the metabolism and bioavailability of phytochemicals

An important function of host-microbial co-metabolism 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 phenolic due to its medicinal properties that are linked to its anti-inflammatory and anti-oxidant activities (Wu et al., 2014). The pharmacological activity is 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 amongst 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 II metabolism. A considerable amount of flavonoid metabolites are 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 the host and microbiota expressed enzymes. 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, Epimedii used to treat osteoporosis (Li et al., 2015). Epimedii 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, question whether conditions of osteoporosis may modulate the metabolism of the major flavonoids present in *Epimedii* in a manner that may ultimately affect its bioavailability and efficacy.

Flavonoids are also important constituents of Astragali Radix, a traditional Chinese herbal medicine used to treat a wide variety of disease states due to 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 pharmacological properties of the most

abundant flavonoid present in Astragali 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*.

The impact of the gut microbiome on the metabolism and pharmacokinetics of drugs and xenobiotics

The gut microbiome utilizes 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 *H. 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 biological activity of drugs and xenobiotics involve reduction, hydrolysis, dihydroxylation, acetylation, deacetylation, proteolysis, deconjugation and deglycosylation processes (Sousa et al., 2008). While 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 below provide a tantalizing glimpse into the emerging role of the gut microbiota in drug metabolism and pharmacokinetics.

Bacterial nitroreduction reactions are of considerable interest as they have been shown to significantly impact the pharmacological 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 CB 1954 (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 that is expressed by *E. coli*. Since nitroreduction leads to the inactivation of nitrobenzodiazepine, it is proposed that NfsB may be useful for developing anti-addictive agents. The anti-cancer drug CB 1954, 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). While 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 BILR 355, an inhibitor of the

human immunodeficiency virus (Li et al., 2012). BILR 355 is extensively metabolized by CYP3A. However, study of the concomitant administration of ritonavir with BILR 355 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.

An 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 II metabolites include glucuronide and sulfate conjugates. In addition, a metabolite has been identified in the feces 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 that have been 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 H_2 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 was found to increase the bioavailability of amidarone, an antiarrhythmic agent (Matuskova et al., 2014). However, it is yet to be determined whether these changes in

the pharmaocokinetics 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 (SS et al., 2014). Of key importance are the sulfate reducing bacteria which via their production of H_2S, 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 and 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 II conjugation with typically glucuronic acid, glutathione and/or sulfate. Recent results obtained using a simulator of the human microbiota indicates 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 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 propellent and in a number of industrial processes and the synthesis agricultural chemicals (Choudhary and Hansen, 1998). Adverse effects associated with human exposures to hydrazines include hepatotoxicity, reproductive and neurological effects and cancer. Hydrazines are subject to acetylation by N-acetyl transferase and oxidation by cytochrome P450s 1A1, 1A2, 2B1 and 2E2. While germ-free rats exhibited greater toxicity in response to a single orally administered dose of hydrazine (60 mg/kg) as compared to 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/inactivation status. This topic is undertaken by (McCabe et al., 2015) in this issue who uses 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 co-metabolism of drugs 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 inter-individual differences in drug responses. While 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 at 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 inter-individual 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 better define its overall impact on patient response, the factors that contribute to inter-individual 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 will also provide new opportunities for exploiting the host-microbiome relationship to develop either more effective or safer therapies.

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Footnote

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