REVIEW

The gut microbiome: an orchestrator of xenobiotic metabolism

Stephanie L. Collins\textsuperscript{a}, Andrew D. Patterson\textsuperscript{b,\*}

\textsuperscript{a}Department of Biochemistry, Microbiology, and Molecular Biology, the Pennsylvania State University, University Park, PA 16802, USA
\textsuperscript{b}Department of Veterinary and Biomedical Science, the Pennsylvania State University, University Park, PA 16802, USA

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\textbf{Abstract}  Microbes inhabiting the intestinal tract of humans represent a site for xenobiotic metabolism. The gut microbiome, the collection of microorganisms in the gastrointestinal tract, can alter the metabolic outcome of pharmaceuticals, environmental toxicants, and heavy metals, thereby changing their pharmacokinetics. Direct chemical modification of xenobiotics by the gut microbiome, either through the intestinal tract or re-entering the gut via enterohepatic circulation, can lead to increased metabolism or bioactivation, depending on the enzymatic activity within the microbial niche. Unique enzymes encoded within the microbiome include those that reverse the modifications imparted by host detoxification pathways. Additionally, the microbiome can limit xenobiotic absorption in the small intestine by increasing the expression of cell–cell adhesion proteins, supporting the protective mucosal layer, and/or directly sequestering chemicals. Lastly, host gene expression is regulated by the microbiome, including CYP450s, multi-drug resistance proteins, and the transcription factors that regulate them. While the microbiome affects the host and pharmacokinetics of the xenobiotic, xenobiotics can also influence the viability and metabolism of the microbiome. Our understanding of the complex interconnectedness between host, microbiome, and metabolism will advance with new modeling systems, technology development and refinement, and mechanistic studies focused on the contribution of human and microbial metabolism.

\textbf{Abbreviations:}  5-ASA, 5-aminosalicylic acid; 5-FU, 5-fluorouracil; AHR, aryl Hydrocarbon Receptor; ALDH, aldehyde dehydrogenase; BDE, bromodiphenyl ether; BRV, brivudine; BVU, bromovinyluracil; CAR, constitutive androgen receptor; cgr, cytochrome glycoside reductase; CV, conventional; CYP, cytochrome P450; ER, estrogen receptor; FXR, farnesoid X receptor; GF, germ-free; GUDCA, glycoursodeoxycholic acid; NSAID, non-steroidal anti-inflammatory drug; PABA, \textit{p}-aminobenzenesulphonamide; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; PD, Parkinson’s disease; PFOS, perfluorooctanesulfonic acid; PXR, pregnane X receptor; SCFA, short chain fatty acid; SN-38G, SN-38 glucuronide; SULT, sulfotransferase; TCDF, 2,3,7,8-tetrachlorodibenzofuran; TUDCA, tauroursodeoxycholic acid; UGT, uracil diphosphate-glucuronosyltransferase.

\textsuperscript{\*}Corresponding author. Tel.: +1 814 867 4565.
E-mail address: adp117@psu.edu (Andrew D. Patterson).

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1. Introduction

Microbes within the gastrointestinal tract, including bacteria, yeast, and viruses, are perhaps the first to interact with ingested xenobiotics. Moving through the stomach to the small and large intestines, the population of bacteria colonizing the intestinal tract exponentially increases from approximately $10^3$ bacteria/mL of ileal contents to $10^{10}$–$10^{11}$ bacteria/g of stool. The quantity and diversity of the gut microbiome is astounding when considering that their cumulative 3.3 million unique genes outweigh human genes by roughly 150 times. This catalog of bacteria is generally represented by the taxa Bacteroidetes, Firmicutes, Proteobacteria, and Verrucomicrobia, but the proportions of these differ significantly between individuals and over time. However, the metabolic pathways represented in the genomes of this community are well-conserved. This functional conservation represents an efficient adaptation that has developed through the coevolution of microbes and host, where particular functions, rather than bacteria, were conserved based on the conditions in the gut. In the case of humans, a symbiotic relationship has formed with the commensal gut microbiome. The nutrient-rich, anoxic environment of the colon supports anaerobes that utilize human-indigestible compounds, while the products made by these organisms have many benefits to the host [e.g., short-chain fatty acids (SCFAs) such as butyrate and acetate], including reducing inflammation and aiding digestion.

Disruptions to the composition and activity of the gut microbiome contribute to a variety of human diseases. An indicator of microbiome health is community diversity, as redundancy in the bacterial genes responsible for metabolizing 271 drugs in 76 representative gut microbes in vitro before extending to the complete microbiome ex vivo and in vivo. These robust techniques allow for thorough examinations of xenobiotic metabolism mechanisms within the gut microbiome. Additionally, improvements in the manipulation of large scale data from these ‘omics’ techniques have been essential to begin to dissect the associations between bacterial metabolism and disease. Thus, there is tremendous interest in understanding the molecular mechanisms behind the communication between the gut microbiome and host that influence the pharmacokinetics of xenobiotics.

The present review seeks to communicate notable findings on the various mechanisms of host-microbiome communication that relate to the metabolism of xenobiotics (summarized in Fig. 1). Xenobiotics are defined as any substances that are not natively produced by the host, but the term is most commonly used to refer to pharmaceuticals, environmental toxicants, and heavy metals. The gut microbiome manipulates xenobiotic metabolism through direct and indirect mechanisms, while simultaneously being shaped by host, dietary, and lifestyle factors. The classic channels of direct xenobiotic metabolism by the microbiome lead to the conversion of chemicals into an inactive metabolite or bioactivation of a prodrug. Xenobiotic absorption in the small intestine is also sensitive to changes in the microbiome because the chemical can be bound to and sequestered by bacteria in the intestine and the host intestinal barrier properties rely on microbial interactions. Lastly, expression of host metabolic enzymes in the liver and intestine, including the cytochrome P450s, conjugative enzymes, and transporters, are influenced by the metabolites secreted by the gut microbiome.

2. The gut microbiome directly metabolizes xenobiotics

Ingested xenobiotics interact with the abundant microbial populations in the small intestine and colon, which often have the capacity to transform them in ways unique or complementary to the host. While human metabolism consists mainly of oxidation, hydrolysis, and conjugation of chemicals with small molecules such as glucuronide or glutathione, the metabolic repertoire of enteric bacteria is much more diverse. The gut microbiome predominantly relies on modification by reduction, addition of acetyl and methyl groups, and radical formation. This has resulted in drugs being metabolized into unexpected products by microbial enzymes, for example, β-glucuronidases, nitroreductases, and sulfoxide reductases. Human metabolism is also complemented and expanded by bacterial enzymes that perform similar functions to host enzymes. This is apparent by the nearly 3000 predicted cytochrome P450 (CYP450) enzymes present in bacteria, compared to the recorded 57 in humans. Much research has been dedicated to understanding how microbes uniquely modify xenobiotics, and the impact these metabolites have on host health. Though it is understood now that the gut microbiome can significantly disrupt or accelerate the pharmacokinetics of...
xenobiotics, screening of microbial drug metabolism has yet to be adopted as part of the drug development process despite high-throughput methods being developed.

### 2.1. Xenobiotic inactivation by the gut microbiome

The metabolism of drugs by microbes in the gut is a concern for therapeutic efficacy and safety and can be challenging to predict in humans. An interesting example of a drug being metabolized by a single bacterium is the cardiac glycoside digoxin, a treatment for heart failure and arrhythmia. Digoxin’s mode of action is through the direct binding and inhibition of Na+/K+ ATPases by its unsaturated lactone rings, which lowers the Ca2+ concentration in cardiac myocytes. In some individuals, digoxin is reduced into metabolites such as dihydrodigoxin, which are inactive due to their saturated lactone rings (Fig. 2A). Gut microbes were discovered to metabolize digoxin with the observations that fecal samples could convert digoxin to dihydrodigoxin, and that administering broad-range antibiotics reversed this activity. Dobkin et al. determined that digoxin reduction could be attributed to a sole bacterium Eggerthella lenta (formerly Enterobacterium lentum). However, colonization of E. lentum in an individual’s microbiome was not enough to predict digoxin inactivation because of strain-level differences in their metabolism. Using transcriptomics and quantitative PCR, it was reported that the cytochrome glycoside reductase (cgr) operon was responsible for the metabolism of digoxin and other unsaturated lactone rings by the select strains of E. lentum that had an intact copy (Fig. 2A). Furthermore, the reductases and drug transporters suggested to metabolize the drug were found to be upregulated with digoxin treatment. Cooperation between members of the microbiome often occurs in the metabolism of xenobiotics, as is the case for the Parkinson’s disease drug levodopa (L-DOPA). The microbiome inactivates L-DOPA, initially by decarboxylation into dopamine by a tyrosine decarboxylase of Enterococcus faecalis, then by dehydroxylation into m-tyramine by a dopamine dehydroxylase of E. lenta A2. These results emphasize how variations in the activity and interactions of the gut microbiome contribute to differences in drug metabolism between individuals and perhaps between animal models and human subjects. Given that bacteria have unique nutritional requirements, manipulations by dietary components can be used to alter microbial drug metabolism. For example, treating E. lentum with arginine, while increasing its growth, inhibits digoxin metabolism by blocking its expression of cgr. With an increased understanding of the mechanism of drug metabolism by the gut microbiome, directed treatments independent of broad-range antibiotics can be developed to improve drug efficacy or reduce toxicity.

Another concern with the microbial metabolism of xenobiotics is drug inactivation, as it reduces drug efficacy and leads to variable responses between individuals. For example, the active concentration of the chemotherapeutic drug gemcitabine is known to be reduced by enteric microbes. Gemcitabine-treated mice intratumorally injected with Gamma proteobacteria developed more colon polyps than their PBS-injected counterparts, and tumor development could be reversed with the antibiotic ciprofloxacin. Pre-incubation of gemcitabine with Escherichia coli was also shown to prevent its efficacy upon administration to BALB/c mice. It was determined that cytidine deaminases present in these organisms were responsible for converting gemcitabine into its inactive metabolite, 2′,2′-difluorodeoxyuridine. Unlike digoxin, whose metabolism occurs through the bacterial-specific cgr operon, cytidine deaminases also exist in human cells as part of the pyrimidine salvage pathway. Thus, the
Figure 2  Examples of xenobiotics metabolized by the gut microbiome. (A) The inactivation of digoxin by reduction using the proteins expressed from the cgr operon only present in *Eggerthella lenta*. (B) Conversion of the prodrug sulfasalazine into the active ingredient 5-aminosalicylic acid (5-ASA) and its toxic by-product sulfapyridine. Later iterations of 5-ASA releasing drugs ipsalazide and balsalazide produce the non-toxic metabolites 4-aminobenzoylglycine and 4-aminobenzylalanine, respectively. (C) Irinotecan (also known as CPT11), once absorbed into the liver, is metabolized into SN-38 and SN-38 glucuronide (SN-38G), of which the latter is mainly secreted back into the intestine through enterohepatic circulation. While SN-38G is not toxic to intestinal cells, β-glucuronidases expressed by the gut microbiome convert it to the cytotoxic SN-38 metabolite.
relative impact of microbiome and host enzymes in the metabolism of gemcitabine is challenging to ascertain and remains uncertain.

One solution to improve the resolution of microbiome and host activities to the metabolism of xenobiotics is through the advancement of global modelling techniques. Recently, Zimmermann et al. used this approach to differentiate mouse and bacterial metabolism of brivudine (BRV), an antiviral drug. Comparing the concentrations of BRV and its metabolite bromovinyluracil (BVU) throughout the digestive tract, germ-free (GF) mice were found to produce 5 times less BVU than conventional (CV) mice. Gnotobiotic mice that specifically lack the BRV-metabolizing \textit{bt}4554 gene in their microbiome, and mice mono-colonized with \textit{Bacteroides thetaiotaomicron}, also lacking \textit{bt}4554, metabolize BRV as poorly as GF mice. This was used to delineate the host contribution to BVU production and BRV/BVU absorption, while forgoing the physiological changes that typically affect GF mice. Using global analysis, kinetic measurements from GF vs. CV and \textit{bt}4554 WT vs. KO experiments were successfully integrated to model all aspects of BRV metabolism (Pearson’s correlation = 0.98), including absorption, intestinal tract migration, and drug conversion by the microbiota and host. The model was validated and expanded to include enterohepatic circulation in experiments using two additional drugs, sorivudine and clonazepam. This study demonstrates that the future of pharmacokinetic modeling will require a consideration of the microbiome’s contributions to metabolism. However, due to the extreme manipulations to the gut microbiome required to control for microbial metabolism, these model systems remain somewhat limited in their applicability to humans.

2.2. Xenobiotic bioactivation by the gut microbiome

While some xenobiotics are inactivated through bacterial metabolism, others can be converted from a precursor (prodrug) to an active metabolite. Many unique active chemicals can be produced by the broad repertoire of enzymes expressed in the gut microbiome that are not present in the host. Since the microbiome also significantly varies in the guts of different species and individuals, drug efficacy can vary between animal studies and human trials, and between individuals. However, the expanded profile of metabolites produced by the gut microbiome can also be a source of new drugs, such as the humimycin antibiotics produced by \textit{Rhodococcus spp.}. These xenobiotic metabolites often have localized effects, either beneficial or detrimental, in the small intestine or colon due to the release of active ingredients where the microbiota is present.

The first report of drug bioactivation by the microbiome was with the bacterial-specific metabolism of the antibiotic prontosil. Although it was inactive \textit{in vitro} against streptococci, prontosil was able to treat infections when administered to mice. It was later discovered that the active ingredient was not prontosil, but its azo-reduced metabolite \textit{p}-aminobenzenesulphonamide (PABA). Since prontosil was only metabolized when it was ingested or injected intravenously, but not subcutaneously, it was proposed that the gut microbiome was responsible for PABA production in the colon, whether directly or through enterohepatic circulation. Other drugs containing azo bonds are similarly reduced by the gut microbiome. The ulcerative colitis drug sulfasalazine is composed of the active ingredient \textit{5}-aminosalicylic acid (\textit{5}-ASA) azo-linked to sulfapyridine, which is released by bacterial metabolism (Fig. 2B). However, due to the toxic hemolytic properties and other dose-limiting side effects of sulfapyridine, alternative drugs have since been designed with \textit{5}-ASA conjugated to \textit{4}-aminobenzoylglycine (ipsalazide) or \textit{4}-aminobenzoyl-\begin{small}$\beta$\end{small}-alanine (balsalazine) (Fig. 2B).

Although expressed in both bacterial and human cells, microbial nitroreductases can significantly impact drug toxicity by modifying the localization and abundance of metabolites in the gut. Several drugs that would normally be bioactivated in low concentrations by nitroreductases predominantly in the liver, are highly converted by the abundance of such enzymes in the gut microbiome. The chemotherapeutic pro-drug CB1954, for example, is bioactivated by \textit{E. coli} Nissle 1917 \textit{in vitro} and in BALB/c mice intratumorally injected with \textit{E. coli}. However, the extent of drug conversion by microbiota in the gut, and not the tumor microenvironment, is currently unknown. Nitrobenzodiazepines are a group of structurally related chemicals that are commonly prescribed as anxiolytics, including nitrazepam, flunitrazepam, and clonazepam. Nitrazepam is converted by nitroreductases in the cecal contents of Sprague–Dawley rats into the teratogen \textit{7}-aminonitrazepam and all three compounds are similarly reduced by the gut colonizers \textit{E. coli}, \textit{E. cloacae}, and \textit{S. typhimurium}. Though nitrazepam can also be metabolized in the liver, treating rats with antibiotics dramatically reduces \textit{7}-aminonitrazepam production, suggesting the importance of enzyme niche and activity, and perhaps the affinity of bacterial enzymes for the drug.

Dietary xenobiotics can also exert their beneficial effects through their bioactivation by the gut microbiome. For example, the digestion of soy-derived isoflavones into the estrogen analogue equol. An initial observation that post-menopausal Japanese women experienced less severe hot flashes than Finnish and American women led to the suggestion that elevated soy intake could mitigate the side effects of menopause. Soy products are a rich source of the isoflavones daidzein and genistein, which are metabolized \textit{in vitro} by enteric anaerobes into equol and \textit{5}-hydroxy-equol, respectively. These, and the secondary metabolites dihydrodaidzein and dihydrogenistein, are bacterial-specific products that beneficially regulate estrogen due to their ability to act as analogues for estrogen receptors (ERS). Interestingly, they can be agonistic or antagonistic to the ER depending on what is needed to balance native estrogen levels. Equol, the compound most associated with beneficial effects, is correlated with certain members of the gut microbiome such that approximately 30%--50% of individuals produce it in measurable amounts. In line with the observation that certain women experience less severe menopause, microbial equol production is consistent over at least several years in women with this activity, enough to cause a long-term benefit.

These studies highlight the importance of identifying the microbial metabolism of drugs, since their active or toxic products can significantly impact how we design and implement drugs on the market. Furthermore, due to the variation in microbial activity between individual patients, drug dosages should account for the potential elevations in active forms of the drugs by the microbiome. Although pharmacokinetic modeling has long been applied to human and animal models, they are only beginning to be translated to microbes, perhaps because of the extreme enzymatic complexity and our relatively limited understanding of their metabolic capacity. However, such advances will be essential and dependent upon studies using metabolomics to identify, quantify, and differentiate the production of metabolites from microbial and host processes.
2.3. Reactivation of host-detoxified compounds by the gut microbiome

An emerging concern with the microbial metabolism of xenobiotics is the reactivation of chemicals that have already been detoxified by host enzymes. Human phase II metabolism relies on the conjugation of xenobiotics or endobiotics with small molecules to alter their excretion. The most prevalent modifications are acetylation, methylation, glucuronidation, sulfonation, and glutathione or amino acid conjugation, which each require cofactors and transferases to deliver small molecules to recipient chemicals. By increasing their size and polarity (except acetylation and methylation), conjugation prevents unregulated passive diffusion of hydrophobic molecules through cell membranes, thereby forcing retention in excretory pathways. Although conjugation can, in very few cases, bioactivate the modified chemical, most xenobiotics at this stage are detoxified. Since these reactions occur predominantly in the liver, xenobiotics are then exported into the blood or the biliary duct. Once they are secreted into the small intestine through enterohepatic circulation, sequestered compounds can be deconjugated by gut microbes. Acetylases, methylases, and glucuronidases are particularly widespread in the gut microbiome, as they are a rich carbon source for energy metabolism. Similarly, glutathione and amino acid deconjugating enzymes free nitrogen to be fed into nitrogen metabolism pathways. For the host, these enzymes can be dangerous as they reactivate detoxified chemicals to maintain elevated concentrations for longer.

Although also present in human cells, bacterial  β-glucuronidase activity in the gut can worsen the side effects of drugs detoxified by glucuronidation, such as the non-steroidal anti-inflammatory drugs (NSAIDs). Irinotecan, also designated CPT11, is a first-line chemotherapeutic used predominantly for colorectal cancer. Its active ingredient is the host bioactivated metabolite SN-38, which is predominantly produced in hepatocytes and delivered throughout the body by blood (Fig. 2C). SN-38 is harmful to the healthy intestinal epithelium in addition to cancer because it targets rapidly dividing cells that are found in intestinal crypts. Thus, prior to biliary excretion, UDP-glucuronosyltransferases (UGTs) in the liver add glucuronic acid to form SN-38-glucuronide (SN-38G), which can pass harmlessly through the gut (Fig. 2C). However, microbiome  β-glucuronidases convert SN-38G back to SN-38, leading to delayed-onset, severe diarrhea that limits the effective dose that can be administered (Fig. 2C). Developments in microbial  β-glucuronidase inhibitors can be used to prevent this side effect. A similar mechanism was identified with NSAIDs, such as diclofenac, which are converted to their aglycone form by bacterial glucuronidases, leading to adverse gastrointestinal effects. With the identification of demethylases, desulfatases, glucuronidases, and other phase II reversing enzymes in the enzymatic arsenal of the microbiota, there is a growing potential for the reactivation of xenobiotics in the gut.

2.4. Summary of direct microbial metabolism of xenobiotics by the gut microbiome

Several examples of the direct metabolism of ingested xenobiotics by the gut microbiome have been presented, leading to the inactivation of active compounds, bioactivation of prodrugs, or production of newly toxic metabolites. Each outcome has significant implications for the pharmacokinetics of xenobiotics, adding to the complicated and variable patient responses to drugs or environmental toxicants. It will be important to test potential therapeutics for their modification by microbial enzymes considering the impact of such alterations to xenobiotic metabolism. One approach to test microbial metabolism of xenobiotics is the application of drugs to in vitro culture, which may be performed on either a sample of the host microbiota or with a subset of representative strains. The former has the advantage of more accurately representing the entire gut microbiome of the individual, but it is more challenging and therefore lower throughput. While culturing individual strains affords more high-throughput potential which is valuable for large-scale drug studies, it may lack translatability due to strain- and species-level variations in metabolism. However, with improvements in pharmacogenomic modeling and knowledge of microbial metabolism, it may eventually be possible to predict xenobiotic alterations by measuring the total genomes (metagenomics) or transcriptomes (metatranscriptomics) of the microbiome. Understanding which genes lead to xenobiotic metabolism from RNA-seq studies of the microbiota in response to drug treatment has improved. It is possible that isolating single cells using flow cytometry and performing RNA-seq on them will help to delineate the relative contribution and response of individual bacterial strains to xenobiotic metabolism. Ultimately, these metabolic studies could provide insight into the variability in drug responses between individuals and in translating animal studies to humans.

3. Disrupted compound absorption by the gut microbiome

The bioavailability of ingested chemicals or their metabolites is influenced by the gut microbiome through modifications to the intestinal mucosal barrier, which is responsible for limiting the absorption of chemicals particularly through the small intestine. It is composed of intestinal epithelial cells associated with tight junctions, immune cells to control bacterial invasion, and the overlying mucus layer that houses the gut microbiome. Thus, there are several avenues through which the microbiome may influence xenobiotic absorption, namely by altering intestinal permeability, thickening the intestinal mucus layer, and physically binding to compounds to prevent their absorption. By redistributing the localization of xenobiotics throughout the body, these are important mechanisms in drug toxicology.

A study by Hayes et al. demonstrated that the microbiome increases paracellular permeability by reducing the tight junction proteins claudin-1 and occludin between intestinal epithelial cells. However, commensal microbes may individually support stronger tight junction formation and some studies have shown an increase in the enterocyte microvilli barrier protein SPRR2A in CV compared to gnotobiotic mice. Additional research further supports the idea that microbes have an overall preventative effect on absorption. The microbiome is essential for shortening enterocyte microvilli and increasing intestinal transit time, which limit the uptake of xenobiotics into circulation. Compared to GF animals, CV animals have a thicker and less permeable intestinal mucus layer. Variations in mucosal thickness are observed with differences in the healthy microbiome, particularly in the abundance of the mucus-degrading bacterium Akkermansia muciniphila. Altogether, these results suggest that the host intestinal barrier is supported by microbiome colonization, potentially leading to the reduced absorption and increased clearance of xenobiotics.

In terms of actual drug absorption studies in GF and CV mice, the absence of a microbiota appears to increase passive transport...
through enterocytes, but not active transport mechanisms\textsuperscript{69,70}. However, heavy metals rely on absorption by protein transporters with cationic affinity, such as calcium transporters or metallothioneins, which are perturbed by the presence of the microbiome\textsuperscript{71}. GF mice, for example, absorb greater concentrations of cadmium and lead into circulation, leading to their accumulation in the kidneys, liver, and spleen\textsuperscript{72}. Conversely, some lipid soluble compounds are more readily absorbed in the presence of the microbiome. Lipid uptake in the small intestine of GF mice is disrupted due to impaired cholecystokinin signaling, which leads to reductions in pancreatic lipase secretion and enterocyte expression of lipid translocators\textsuperscript{73}. Variations in the gut microbiome of zebrafish, particularly in the levels of Firmicutes, are associated with increased lipid absorption into the gut epithelium\textsuperscript{74}. Thus, these observations may not be limited to the extreme cases of GF animals, but also observed between normally colonized individuals.

While the gut microbiome can influence absorption indirectly through alteration of the host intestinal barrier, it may also sequester absorption through direct binding of xenobiotics in the lumen. An early example of this was seen with the Parkinson's disease (PD) treatment L-DOPA, which is converted to dopamine in the central nervous system, thereby helping to alleviate tremors. It was initially observed that PD patients treated with L-DOPA had lower concentrations of the drug in their blood if they were populated with Helicobacter pylori, and this difference was ameliorated with antibiotic treatment\textsuperscript{75,76}. Later, it was discovered that L-DOPA adsorbs to the surface of H. pylori in vitro via bacterial adhesins\textsuperscript{77}. Thus, the adherence of xenobiotics to bacterial surface proteins may prevent absorption into circulation. Various species of Lactobacillus can prevent the absorption of the fungal toxins aflatoxin B\textsubscript{1}\textsuperscript{78,79} and zearalenone\textsuperscript{80}, as well as heavy metals\textsuperscript{79,81}, by xenobiotic binding to the bacterial surface. Given the widespread concern for their toxicity, this concept has been successfully adopted to develop probiotics for the prevention and remediation of toxic metal exposure in humans\textsuperscript{82}. Lastly, some xenobiotics, such as cadmium, are prevented from absorption by being imported into bacteria of the gut microbiome, such as E. coli\textsuperscript{83}.

The bioavailability of xenobiotics can be influenced by altered host intestinal permeability, mucus layer thickness, or through direct interaction of compounds with bacteria. To date, microbial influences on absorption have been underappreciated in xenobiotic metabolism models. Additional research must be done outside of GF animal studies, as GF mice are not good representations of the human gut. It is important to appreciate that small differences in absorption can elevate local concentrations of xenobiotics into the zone of toxicity, particularly if they are bioaccumulated, or lower circulating concentrations of pharmaceuticals to prevent their effectiveness.

4. Host xenobiotic metabolism enzymes are influenced by the gut microbiome

The microbial community can significantly impact host expression of genes in a variety of metabolic pathways, whether proximally in intestinal epithelium or in distal organs such as the liver\textsuperscript{84}. These distal interactions are made possible by the uptake and delivery of metabolites that are produced by microbes in the gut. The current dogma of the symbiosis of humans and microbes is that the stimulation of physical and immune defenses by the microbiome primes the body against insult by pathogens. However, the concept can be applied to xenobiotic metabolism, as the gut microbiome appears to promote the expression of enzymes primarily involved in detoxification, leading to a priming of the host against xenobiotic exposure. Alterations to xenobiotic metabolism by the microbiota have been observed at all levels, including nuclear receptor regulation, expression of proteins involved in phase I (oxidation) and phase II (conjugation) metabolism, expression of ABC-like transporters, and functional differences in metabolites. Thus, the microbiome acts to prime the host to detoxify xenobiotics by manipulating the profile of metabolic enzymes available.

4.1. Microbiome alters xenobiotic nuclear receptor activity

Nuclear receptors are the master regulators of gene expression, particularly for enzymatic activity in the liver and intestine, where they are predominantly expressed. Upon activation by ligand binding, nuclear receptors dimerize and bind promoter regions of essential metabolic genes to recruit RNA polymerase II for transcription\textsuperscript{85}. Among the repertoire of genes under their control are CYP450s, responsible for oxidation—the first phase of classical xenobiotic and endobiotic metabolism. Several phase II enzymes and transporters, including UGTs, sulfotransferases (SULTs), methyltransferases, and multi-drug resistance genes, are also under nuclear receptor control. The nuclear receptors pregnane X receptor (PXR), constitutive androgen receptors (CAR) 1–3, and farnesoid X receptor (FXR), and the PAS domain protein aryl hydrocarbon receptor (AhR), bind xenobiotics that induce or inhibit the expression of their target xenobiotic metabolism genes. PXR is particularly important as it controls the expression of CYP3A4, the enzyme known to perform oxidation on approximately 50% of all drugs\textsuperscript{86}. Furthermore, PXR has a large, flexible ligand-binding domain that allows it to respond to a broad assortment of xenobiotics\textsuperscript{85}. Thus, there is potential for the microbiome to regulate xenobiotic metabolism through the promiscuity of ligand activated transcription factors.

Through comparisons with GF animals, the presence of the gut microbiome appears to have mixed effects on the expression and activation of xenobiotic transcription factors. In NRM1 and C57BL/6J mouse liver and colon, CV or SPF conditions reduce CAR expression compared to GF mice\textsuperscript{87–89}. CAR expression increased in a study using SPF IQI/Jic mice\textsuperscript{90}, though this strain is a model for the autoimmune disease Sjögren’s syndrome\textsuperscript{91}, which could affect transcription of CAR. However, CAR mRNA levels are no different between SPF and GF in another study using C3H/OrlJ mice\textsuperscript{92}. Conflicting results have also been observed for other xenobiotic nuclear receptors and transcription factors. CV mice, compared to GF, have higher levels of AhR, FXR, and PXR on an IQI/Jic mouse background\textsuperscript{93}. In other studies, AhR and PXR were suppressed in C57BL/6J mice\textsuperscript{94} and PXR was unchanged in NMRI mice\textsuperscript{95}. It is therefore difficult to discern what the true impact of the microbiota is on nuclear receptor expression, as several factors may be responsible for the varied observations. First, the mouse strain used in each study could impact expression profiles. The age of mice also makes a significant difference, as seen in a study by Selwyn et al.\textsuperscript{96}, where AhR and PXR were only differentially expressed between GF and CV mice after 90 days. Even if it is unclear whether the expression of ligand activated transcription factors is altered by the microbiota, ultimately it is the potential of the microbiome to regulate
downstream xenobiotic metabolism genes that is important. In a study by Claus et al., AHR, CAR, FXR, PPARα, PXR, and RXRα expression were the same for GF and CV C3H/Orl mice, but CYP450 genes under their regulation (Cyp2c29, Cyp3a11, and Cyp8b1) were suppressed in GF conditions. Interestingly, conventionalization of these GF mice resulted in the induction of CAR, FXR, and PXR expression compared to CV mice, thus supporting the idea that bacterial metabolites can promote nuclear receptor expression. It is therefore essential that studies identifying associations between the microbiome and nuclear receptor activity perform both activity and expression assays to capture the true functional consequences. Furthermore, it is recommended that nuclear receptor-humanized mice be utilized, due to the differing ligand binding domain specificities between mouse and human receptors.

4.2. Microbiome alters phase I oxidative enzyme expression

Across studies comparing GF and CV mice, many of the largest transcriptomic and proteomic perturbations in the liver occur in CYP450 enzymes. The presence of the gut microbiome in several mouse strains increases hepatic CYP450 expression, which supports the concept that microbes prime the host for xenobiotic metabolism. The CYP3A family makes up approximately 30% of all CYP450 enzymes in the liver but is thought to be responsible for half of all xenobiotic oxidation reactions in the liver and intestine, making it essential for the detoxification of a broad range of compounds. CYP3A4 (CYP3A11 in mice) has been especially implicated in the first-pass metabolism of many xenobiotics, for example, the highly carcinogenic fungal metabolite aflatoxin B1, various antibiotics, acetaminophen, and the chemotherapeutic irinotecan. Across many GF mouse studies, the mRNA, protein, and activity of CYP3A11 is ubiquitously and significantly suppressed in the absence of a native microbiome. Its expression is dependent upon the activation of PXR or FXR by their respective ligands, and subsequent recruitment of RNA polymerase II to the promoter region approximately 90bp upstream of the Cyp3a11 start codon. CYP3A11 expression and activity may then be restored following two months of conventionalization of these GF mice, thus providing an overall reduction in phase II metabolites that occurs in GF mice. Using mass spectrometry to globally identify neutral losses, Wikoff et al. revealed an overall reduction in phase II conjugation of serum metabolites (by, for example, sulfates, glycines, glucuronides) in mice lacking a microbiome. While some metabolites were significantly lowered in GF mice (hippuric acid benzoate/glycine and the glucuronide adduct of 3-carboxy-4-methyl-5-pentyl-2-furanpropionic acid), many others were completely abolished (phenylsulfate, p-cresol sulfate, equol sulfate, cinna-moylecyclohexanecarboxylate, and phenylpropionylglycine). However, UGTs have been observed to increase in expression in GF mice compared to SPF mice, though this is only one level of regulation that could influence overall glucuronidase activity. An evaluation of protein concentration and activity of UGTs in GF and CV mice will be necessary to elucidate the functional consequence of altered UGT expression. The final step of conventional xenobiotic metabolism is the export of modified compounds for excretion using cell membrane transporters. The expression of organic anion transporter-2 (Oatp2), organic cation transporter-1 (Oct1), sodium-taurocholate co-transporting polypeptide (Ntcp), and multidrug resistance-associated protein 3 (Mrp3) are increased in SPF mice, compared to GF, likely because they are regulated by PXR.

4.3. The microbiome alters expression of phase II enzymes and transporters

The global suppression of phase I oxidation in GF animals and with antibiotic treatment is also observed for conjugation reactions and transporter function. The expression of glutathione-S-transferases (Gstpi, Gstm1/2/3, and Gsto1) and sulfoconjugates (Sult1d1, Sult5a1, and Paps2) are broadly upregulated in mice with a complete gut microbiome, compared to their GF counterparts. This is only a small reflection of the much larger reduction in phase II metabolites that occurs in GF mice. Using large-scale microbiome data, it has been shown that microbiotropic xenobiotics might alter the function of CYP450 enzymes. Rats treated with the antibiotic ciprofloxacin have reduced expression of Cyp3a1 (CYP3A4 homolog) and Cyp2c11 (CYP2C9 homolog), potentially through its reorganization of the gut microbiome. In humans treated with the broad-spectrum antibiotic clarithromycin, intestinal CYP3A4 and CYP3A5 activities were significantly depleted with no change in their protein expression. These results, however, are confounded because macrolide antibiotics such as erythromycin and clarithromycin directly inhibit CYP3A4, while rifampin can increase CYP3A4 expression in vitro. Thus, future work is needed to distinguish whether CYP450 activity is due to an altered microbiome or direct activation of xenobiotic nuclear receptors. Such studies could administer microbial modifiers that are not substrates for CYP450 enzymes to mice or humans and observe CYP450 activation, or make associations of CYP450 activity with large-scale microbiome data.

The microbiome and microbial-specific metabolites have also been shown to influence xenobiotic metabolism enzymes apart from the CYP3A family. Members of the CYP450 enzyme families 1–4 are responsible for the oxidation of about 60% of all xenobiotics, such as CYP1A and 1B, which are primarily involved in metabolizing polyyclic aromatic hydrocarbons (PAHs). The microbiome-derived ellagic acid metabolites, urolithin-A and -B, upregulate CYP1A1 and 1B1, while down-regulating CYP3A5 in Caco-2 cells. Other non-CYP450 oxidative enzymes may also be influenced by the microbiota, such as the aldehyde dehydrogenases (ALDHs) that convert aldehydes into carboxylic acids. GF mice have elevated Aldh1b1 and decreased Aldh1a1 and Cyp3a2 expression compared to their GF counterparts. In these cases, little is known about the extent that normal variations in the gut microbiome affect metabolic activity or what the consequences are for the metabolism of specific xenobiotics that are their substrates for these enzymes.
application of bacterial-specific metabolites to CV animals. Urolithins, which are specifically produced by the gut microbiome, elevate UGT1A10 expression and downregulate SULTs in a human epithelial cell line. This demonstrates that the composition of an individual’s microbiome, perhaps even between healthy individuals, may result in differences in expression of proteins essential for drug metabolism.

4.4. The gut microbiome affects host metabolism of xenobiotics

Numerous studies have demonstrated the potential of the gut microbiome to modify the expression of host xenobiotic metabolism enzymes; however, few studies have extended this to evaluate changes to the metabolism of specific xenobiotics. Some microbiome-associated metabolites can inhibit the metabolism of xenobiotics by host enzymes by directly competing for their activity. For example, the bacterial metabolite p-cresol interferes with sulfate conjugation of xenobiotics by SULT1A1 by occupying this enzyme in its conversion to p-cresol-sulfate. It is thought that p-cresol acts through direct competition with substrates of SULT1A1 at its active site and/or by depleting 3'-phosphoadenosine-5'-phosphosulfate, the limiting source of useable sulfate in cells. For drugs requiring sulfate conjugation, such as acetaminophen, the presence of p-cresol can act as a biomarker to predict inhibition of their metabolism. Interference of host xenobiotic metabolism by gut microbiome metabolites can lead to dangerous and unexpected drug interactions, as was observed with sorivudine and the chemotherapeutic drug 5-fluorouracil (5-FU). Sorivudine, an antiviral drug used against varicella-zoster virus and herpes simplex virus type-1, led to 18 mortalities and numerous morbidities in Japanese cancer patients already being treated with 5-FU. Orally-administered sorivudine is dephosphorylated to bromovinyluracil (BVU) by Bacteroides species that are broadly abundant in the gut microbiome. It was discovered that BVU inhibits dihydroprymidine dehydrogenase, the host enzyme responsible for metabolizing 5-FU, causing toxic accumulations of the drug in these patients. Although it may be challenging and time-consuming to evaluate each microbial product of drug metabolism and its effects on host detoxification of other xenobiotics or endobiotics, this can be critical for knowing the adverse effects of pharmaceuticals on the market.

The manipulation of host xenobiotic metabolism by the gut microbiome is often mediated through TLR2, a cellular receptor that is activated by bacterial membrane components and is essential for the intestinal expression of CYP1A1. For example, a study by Do et al. showed that Tlr2-knockout mice are unable to detoxify benzo[a]pyrene, as it relies on oxidation by CYP1A1. Future work should solidify the connection between the gut microbiome and Tlr2-mediated benzo[a]pyrene metabolism, perhaps through observations of its degradation with broad-spectrum antibiotic treatment, using GF animals, or administration of individual bacterial strains known to activate TLR2. Colonic expression of the multi-drug transporter ABCB1/MDR1, required for the cellular export of the chemotherapy drug methotrexate, is promoted by the microbiome through the activation of TLR2. Depleting the microbiome using antibiotics causes severe intestinal damage and mortality in mice administered methotrexate, which is reversed by treating with TLR2 agonists. With a growing interest in understanding the host-microbiome metabolism relationship, more studies are required to explicitly link the metabolism of xenobiotics of interest with microbial activity and alterations to host metabolic enzymes.

5. Xenobiotics modify the structure and activity of the microbiome

An important component of the host-microbiome-xenobiotic metabolism relationship is the reshaping of gut microbiome structure and activity by administered xenobiotics. This has been well-documented for antibiotics, as it is their intended activity to selectively kill microbes. However, antibiotics appear to have longer extended effects on the microbiome than was originally anticipated, which consequently increases resistant bacterial populations long after the selective pressure of antibiotic has been relieved. Even microbes that are not targets of the antibiotic can become damaged and alter their transcription of genes that facilitate protection against xenobiotics, such as multi-drug transporters. It is still unclear how long-term changes to the microbiome’s metabolism and viability affect host health or their ability to tolerate later challenges with xenobiotics. Furthermore, the toxic effects of pharmaceuticals not intended as antibiotics can put selective pressure on the microbiome, causing the expression of antibiotic resistance genes (e.g., the drug efflux transporter tolC). This raises serious concerns about the development of antibiotic resistance from non-antibiotic drugs.

The selective toxicity of xenobiotics to the microbiome perturbs both their physiology and many of their important metabolic functions. Berberine, a natural compound extracted from plants, is required to be chemically converted to its readily absorbed form by the microbiome in order to exert its glucose and lipid-regulating effects. However, it also suppresses the bacterial population important for metabolizing tauro-conjugated bile acids. In turn, the accumulation of unconjugated bile acids activates FXR, a nuclear receptor primarily responsible for the regulation of bile acid metabolism that binds both endogenous and xenobiotic ligands. FXR has downstream effects on gut microbiome viability, and bile acid, lipid and glucose homeostasis. Interestingly, the type 2 diabetes medication metformin suppresses FXR activity through its toxicity to members of the gut microbiome. Metformin selectively depletes Bacteroides fragilis, a bacterium essential for deconjugating the bile acids glyco- and tauro-sodiumxycholic acid (GUDCA) and tauro-sodiumxycholic acid (TUDCA). The subsequent accumulation of GUDCA and TUDCA inhibits FXR signaling in the ileum, further supporting a gut microbiome-related mechanism. Attenuation of FXR signaling has also been observed from the treatment of mice with the environmental toxicant 2,3,7,8-tetrachlorodibenzofuran (TCDF), perhaps by reshaping the gut microbiome to a lower Firmicutes/Bacteroides ratio. Thus, it is apparent that xenobiotic alterations to the microbiome regulate both the microbes’ and host’s abilities to metabolize several endobiotics or xenobiotics that can be distinct from the initial insult.

There is often a dynamic, bidirectional relationship between the metabolism of a xenobiotic and the vitality of the gut microbiome. The heavy metal arsenic has serious health concerns worldwide due to its acute toxicity in humans, as well as its danger as a recognized carcinogen. Oral exposure to arsenic significantly reduces Firmicutes populations and perturbs the metabolome of the gut, presented as lowered acyl carnitine and daidzein production, increased indole production, and disrupted bile acid metabolism. Certain members of the microbiome metabolize common arsenic-containing compounds in vitro, but this produces bioactive sulfate-reduced metabolites. Nevertheless, in vivo, the presence and composition of the gut microbiome has proven to be essential in mitigating arsenic
toxicity. The interaction of arsenic and microbiome is intricate, and it is unknown how exposure to various arsenic compounds changes the microbiome’s metabolic response or which specific microbes are involved in its detoxification.

Apart from limiting the metabolism of additional xenobiotics, altering the microbiome itself can have negative consequences on human health. This is especially true for infants, whose normal gut microbiome development is essential to a functional immune system as an adult, for example.[12,23,132] Some environmental toxicants can be passed to young children from breast milk, including polychlorinated biphenyls (PCBs), heavy metals, and pesticides.[132] Infant exposure to perfluorooctanesulfonic acid (PFOS), PCB-167, and PBDE-28 through breast milk have been shown to perturb microbiome diversity and function, attenuating the production of beneficial SCFAs.[134] The same study showed a negative correlation between PCB-167 levels in infants and overall bacterial metabolism, though it remains to be shown whether this change affects xenobiotics.[134] In adults, it is clear that ingestion of a variety of pollutants, including PAHs, dioxins, TCDD, pyrethroids, and organophosphates, can be detrimental to host health because of their toxicity to the gut microbiome and disruption of xenobiotic metabolism.[135] Some xenobiotics, while not altering bacterial viability or membrane integrity, still increase the expression of xenobiotic metabolism pathways.[34] The bromodiphenyl ethers (BDE) BDE-47 and BDE-99 are widespread environmental pollutants that activate the xenobiotic nuclear receptors CAR and PXR and increase mRNA expression of hepatic CYP450s, SULTs, GSTs, UGTs, and transporters in mice.[138] However, the presence of gut microbiota partially mitigates the dramatic increase in xenobiotic metabolism transcripts from BDE-47 and BDE-99 treatment.[138] Thus, we should critically evaluate how ingested xenobiotics impact the microbiota, both in composition and activity, in addition to how they are modified by microbes.

6. Conclusion and future directions

The multifaceted interplay of gut microbiome, host factors, and xenobiotic metabolism is complex. The repertoire of enzyme reactions available to the microbial world has widened our view of how xenobiotics are metabolized. The products of these microbial enzymes can exert new activities whether they are unique from host metabolites or add to those that are already produced. Microbial xenobiotic metabolism can lead to bioactivation, detoxification, or in some cases like with glucuronidases, it may even reverse host detoxification. The positioning of the microbiome at enterocytes can prevent absorption by binding or importing xenobiotics or by strengthening the intestinal mucosal barrier. Lastly, we are beginning to appreciate that the presence of the microbiome alters host xenobiotic metabolism enzymes to change the fate of endobiotics or xenobiotics typically converted by these pathways.

Increasing our understanding of microbiome interactions with xenobiotic metabolism will improve pharmacokinetic and drug interaction predictions, and potentially lead to personalization of treatment based on an individual’s microbiome composition and activity. The former is dependent on developing technologies and modeling software to accurately describe the complex relationship between host and microbial metabolism and viability. A recent development to this end used global analysis to model the pharmacokinetics of BRV and delineate the contribution of host and microbe to its degradation.[40] Future iterations should focus on modeling more complicated multi-enzyme reactions. A particular challenge will be to model the pharmacokinetics of microbial deconjugation/host conjugation relationships, such as the β-glucuronidases/UGTs, to understand the distribution and concentration of multiple forms of the xenobiotic throughout the body. A promising new complementary approach is the human-on-chip technology, which aims to establish a functional whole human model of metabolism by including an interconnected network of in vitro liver, intestine, and kidney. Each “organ” contains cultured human cells separated from an interconnected network of artificial vasculature by a collagen layer and can include manipulable physical characteristics such as flow and peristalsis.[139] In terms of modeling metabolism along the host-microbiome axis, the mock-intestine can be colonized with an individual’s gut microbiome and challenged with various xenobiotics. Advances in these technologies will improve predictions about individual responses to xenobiotics and our ability to design effective and safe pharmaceuticals.

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Author contributions

Stephanie L. Collins was responsible for original draft and visualization. Andrew D. Patterson was responsible for review and editing, supervision, and funding acquisition.

Conflicts of interest

The authors declare no conflicts of interest.

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