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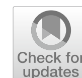

Contribution of the Gut Microbiome to Drug Disposition, Pharmacokinetic and Pharmacodynamic Variability

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Abstract
The trillions of microbes that make up the gut microbiome are an important contributor to health and disease. With respect to xenobiotics, particularly orally administered compounds, the gut microbiome interacts directly with drugs to break them down into metabolic products. In addition, microbial products such as bile acids interact with nuclear receptors on host drug-metabolizing enzyme machinery, thus indirectly influencing drug disposition and pharmacokinetics. Gut microbes also influence drugs that undergo enterohepatic recycling by reversing host enzyme metabolic processes and increasing exposure to toxic metabolites as exemplified by the chemotherapy agent irinotecan and non-steroidal anti-inflammatory drugs. Recent data with immune checkpoint inhibitors demonstrate the impact of the gut microbiome on drug pharmacodynamics. We summarize the clinical importance of gut microbe interaction with digoxin, irinotecan, immune checkpoint inhibitors, levodopa, and non-steroidal anti-inflammatory drugs. Understanding the complex interactions of the gut microbiome with xenobiotics is challenging; and highly sensitive methods such as untargeted metabolomics with molecular networking along with other in silico methods and animal and human in vivo studies will uncover mechanisms and pathways. Incorporating the contribution of the gut microbiome to drug disposition, pharmacokinetics, and pharmacodynamics is vital in this era of precision medicine.

1 Introduction

The goal of precision medicine is to utilize an individual's genetic, environmental, and lifestyle characteristics to ensure appropriate drug therapy and disease state management. Understanding the factors that contribute to the variability in pharmacokinetics and pharmacodynamics is paramount. For example, progress has been made in determining genetic variability in drug-metabolizing enzymes, drug transporters, and drug target genes, resulting in clinically actionable guidelines for select drugs [1]. The gut microbiome with its trillions of microbial cells including bacteria, viruses, fungi, and archaea has recently emerged as an important contributor to drug action and variability, particularly with orally administered compounds. Genes encoding organisms in the human gut microbiome in recent estimates number at 232 million [2], far outnumbering human germline genes of ~ 20,000 [3]. More than 90% of the gut microbiota are members of two bacterial phyla, Bacteriodetes and Firmicutes [4]. The enormous inter-patient diversity in human gut microbiomes and inter-related factors such as diet, circadian rhythms, and immune function are significant contributors to variability in drug disposition and response. Additionally, intra-individual variability across time and influences such as diet are also important considerations when determining relationships between the gut microbiome and drugs [5].

There is a bidirectional nature to the interaction between drugs and the microbiome. Antibiotics, particularly those that impact Gram-positive organisms and anaerobes, can profoundly alter the microbial composition. Some evidence suggests that antibiotic use in infants may change the microbiome ontogeny [6–8] and lead to long-term adverse immunological, neurological, and metabolic outcomes [6–8]. In addition, a significant number of non-antibiotic compounds can alter the gut microbiome with up to 240 drugs showing inhibition of at least one bacterial strain in vitro [9]. This may have implications on antibiotic resistance and dysbiosis-induced disease from traditionally categorized

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Key Points

The gut microbiome is emerging as an important contributor to drug pharmacokinetics and pharmacodynamics interacting with drugs by directly metabolizing them, indirectly affecting host drug-metabolizing enzymes, and modifying the response to drugs

We summarize clinically important interactions between the gut microbiota and several drugs such as digoxin, irinotecan, immune checkpoint inhibitors, levodopa, and non-steroidal anti-inflammatory drugs

non-antibiotic drugs. Conversely, microbes in the gut can modify drugs prior to entering the systemic circulation, thus affecting oral relative bioavailability (F). Microbes and their products also play a role in the gut-liver crosstalk affecting enterohepatic recycling, intestinal and hepatic drug metabolism, and transporters affecting total body drug exposure.

While knowledge of direct microbial drug metabolism has been known, with an azo bond cleavage by colonic bacteria activating the antibiotic sulfasalazine [10], more recent investigations demonstrate that microbes indirectly influence drug action by altering host drug metabolism/transport [11–13]. Microbial products such as bile acids and indoles can alter drug-metabolizing enzyme and transporter activity [14]. Technological advances such as metagenomic sequencing of gut microbes have provided insight into the functions and variability of gut microbes; however, our knowledge is limited and data in humans are evolving. This review focuses on the impact of the gut microbiome on drug disposition and the pharmacokinetics and pharmacodynamics for select drugs. Digoxin, irinotecan, immune checkpoint inhibitors, levodopa, and nonsteroidal anti-inflammatory drugs affected by microbiome alterations with potential clinical impact will be highlighted.

2 Absorption

An orally administered compound must clear several hurdles on its way through the gastrointestinal tract into the portal vein and through the liver before ultimately reaching the systemic circulation. These barriers include physicochemical barriers, transporters, metabolizing enzymes, and bacteria (Fig. 1). Bioavailability variability as a result of these factors is a major contributor to therapeutic failure and/or toxicity. Drugs and other orally administered substances may be metabolized directly by bacteria and bacteria may interact with host-metabolizing enzymes and transporters to alter their activity and thus indirectly affect metabolism. Bacteria may metabolize drugs before absorption, after absorption through the intestinal epithelia, or after biliary excretion from the liver, which may then lead to reabsorption of the drug through enterohepatic recycling.

2.1 Direct Metabolism of Drugs by Bacteria

Bacteria have distinct types of reactions compared to the human host. Bacteria metabolize drugs into more hydrophobic compounds potentially increasing toxicity, while the goal of host metabolism is to metabolize drugs into more hydrophilic compounds decreasing toxicity and facilitating excretion [15]. Modifications performed by bacteria include reduction, hydrolysis, hydroxylation, dihydroxylation, dealkylation, and rarely oxidation. They also can remove functional groups such as N-oxide cleavage, proteolysis, and deconjugation [16]. These bacterial modifications are distinct from those performed by the host cytochrome P450 (CYP) system, which traditionally include N-oxidation and S-oxidation, N-dealkylation and O-dealkylation, aromatic hydroxylation, deamination, and dehalogenation [17].

Gut bacteria directly metabolize a variety of drugs [18]. In many cases, the bacterial species responsible for the drug modification is unknown. In a few cases, extensive investigation has been conducted to determine the species and even strain level. Direct microbe modification of drugs that may be clinically relevant is displayed in Table 1. For example, the cardiac glycoside, digoxin, has been shown to be directly inactivated by the gut microbe *Eggerthella lenta*, leading to the potential for variability in drug concentrations and toxicity in this narrow therapeutic range drug [19]. Recently, another narrow therapeutic range drug, tacrolimus, an immunosuppressive agent used in transplantation, was shown to be linked to *Faecalibacterium prausnitzii*. Kidney transplant patients who required higher doses of tacrolimus had increased amounts of *Faecalibacterium prausnitzii*, a non-motile Gram-positive bacterium present in the gut microbiome [20]. Further investigation showed that incubation of tacrolimus with *F. prausnitzii* produces a keto-reduction product of tacrolimus that was not found when incubated in hepatic microsomes, suggesting a direct biotransformation by gut microbes [21].

2.2 Indirect Metabolism of Drugs by Gut Bacteria

Animal and human studies have provided preliminary evidence that the gut microbiome affects the regulation and activity of metabolizing enzymes. Animal studies have demonstrated that gene expression, protein levels, and activity of drug-metabolizing enzymes are altered in germ-free mice (i.e., mice without a microbiome). *CYP3A* gene

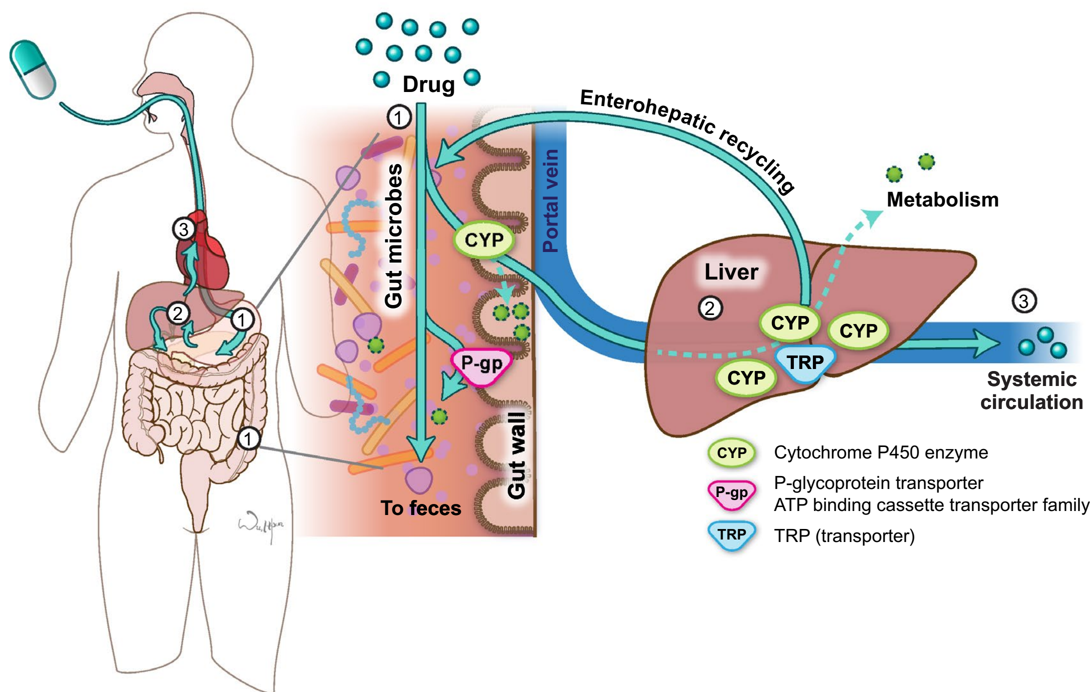

Fig. 1 Pathway of an orally administered drug. When a drug is administered orally (1), it encounters gut microbes, cytochrome P450 (CYP) enzymes, and transporters (TRP) such as P-glycoprotein (P-gp) in the small and large intestine. Some drug will be lost in the feces in these processes. The drug that survives the small intestine will then travel through the portal vein to the liver where it encounters more CYPs and TRP and more drug may be lost to metabolism (2). Some drugs undergo enterohepatic recycling in which drug conjugates are transported from the liver back to the intestine where they encounter microbes, CYP, and TRP again. The amount of drug that enters the systemic circulation is a fraction of what was originally ingested (3). *ATP* adenosine triphosphate

expression was shown to be markedly downregulated in germ-free mice. This was correlated with decreased pregnane X receptor (PXR) binding, a known transcriptional upregulator of CYP3A in the liver [11]. Conventionalization of the germ-free mice restored CYP3A to near-normal levels [12]. Treatment of conventional mice with the quinolone antibiotic ciprofloxacin caused decreased hepatic CYP3A expression and decreased metabolic activity of the CYP3A substrate triazolam; however, no changes were seen when ciprofloxacin was given to germ-free mice [13]. This suggests that microbes or microbial products may bind to nuclear factors such as PXR to downregulate the expression of drug-metabolizing enzymes such as CYP3A.

There are sparse human data but the results are concordant with animal results (Table 2). A study in healthy volunteers showed decreased CYP1A2, CYP2C19, and CYP3A4 activity after a 7-day course of the second-generation cephalosporin, cefprozil [33]. Enzyme activities were measured using a modified Cooperstown cocktail [34] of caffeine for CYP1A2, omeprazole for CYP2C19, and midazolam for CYP3A4. Analysis of the microbial community showed decreased alpha diversity and a correlation between loss of alpha diversity and increased drug and metabolite formation for all three probe compounds [33]. Altering the microbiome with antibiotic therapy modestly decreased enzyme activity, which suggests that a healthy and diverse microbiome may be necessary for optimal functioning of drug-metabolizing enzymes. Future investigations into the mechanism of this effect as well as with other antibiotics will provide additional clinically actionable information.

3 Enterohepatic Recycling

Enterohepatic recycling occurs when xenobiotics or endogenous substances are absorbed through enterocytes, processed by hepatocytes, then secreted into the bile where they are then reabsorbed by intestinal cells. Enterohepatic recycling can often be accompanied by hepatic conjugation and intestinal deconjugation. This process can occur continuously and results in a longer mean residence time including multiple peaks during a single-dose concentration vs time profile. Many drugs and endogenous

Table 1 Direct metabolism of drugs by gut microbes associated with pharmacokinetic changes

Drug	Animal/human	In vivo/in vitro	Organism	Effect on PK (intact gut microbiome)	Comments
Amlodipine [22]	Both	In vivo	N/A	↓ F, ↓ T_{max}, ↓ C_{max}, ↓ AUC	Antibiotics may increase bioavailability by suppressing gut microbial metabolic activities
Aspirin [23, 24]	Animal	Both In vivo	*Lysinibacillus sphaericus*	↓ C_{max} and AUC ↓ F	Reduced gut microbial ASA-metabolizing activity by 67% in rats ↑ F in microbiota-depleted mice
Diclofenac acyl glucuronide [25]	Animal	Both	*Escherichia coli*	↓ C_{max}	*E. coli* β-glucuronidase catalyzed the deconjugation of diclofenac acyl glucuronides in vitro
Digoxin [19]	Both	Both	*Eggerthella lenta*	↑ F	*E. lenta* reduces Cgr 2 → less reduction of digoxin
Indomethacin [26]	Human	In vitro	*Enterobacteriaceae*	↓ Concentrations	β-glucuronidase expression by human gut *Enterobacteriaceae*
Irinotecan [27]	Both	In vitro	*Bacteroides uniformis* and *E. coli*	↑ Abundance (SN-38)	Gut microbial enzymes promote drug toxicity by hydrolyzing the inactive drug → active drug
Levodopa [28–30]	Both	In vivo	*Enterococcus faecalis* and *E. lenta*	↓ Levodopa concentration	Microbes cause less levodopa to be available to cross the blood–brain barrier
Lovastatin [31]	Both	In vivo	N/A	May increase C_{max}, T_{max}, and AUC of M8	The gut microbiota is involved in the metabolism of lovastatin to its bioactive metabolite (M8)
Nabumetone [32]	Animal	Both	*E. coli*	↓ AUC, C_{max}, and half-life	*E. coli* converts the NSAID to a reduced pharmacologically inactive metabolite
Tacrolimus [20]	Human	In vivo	*Faecalibacterium prausnitzii*	Positive correlation b/w *F. prausnitzii* abundance and tacrolimus dose	Study did not explore the potential mechanisms by which *F. prausnitzii* may have influenced tacrolimus metabolism
Tacrolimus [21]	Human	In vitro	*F. prausnitzii* and *Clostridiales* order	Tacrolimus → M1 ↓ F	*F. prausnitzii* may metabolize tacrolimus into M1 (five-fold less potent than tacrolimus as an immunosuppressant)

ASA aspirin, *AUC* area under the concentration–time curve, *b/w* between, *Cgr2* cardiac glycoside reductase operon, C_{max} maximum concentration, *F* relative bioavailability, *M1* tacrolimus metabolite, *M8* lovastatin metabolite, *N/A* not available, *NSAID* non-steroidal anti-inflammatory drug, *PK* pharmacokinetics, *SN-38* active metabolite of irinotecan, T_{max} time to maximum concentration, ↓ decreased

substances are modified by phase II enzymes such as UDP glucuronosyltransferases (UGT), which adds a glucuronic acid moiety to make a more water-soluble metabolite that is more easily excreted into urine or bile. These metabolites often undergo enterohepatic recycling, being secreted into the bile and transported back into the intestine. In the intestine, they can encounter bacterial enzymes such as β-glucuronidase, β-glucosidase, demethylase, desulfurase, and other enzymes with phase II reversing activity that cleave off the small molecules such as glucuronide and make them available again for reabsorption.

The clinical implications of gut bacterial involvement in enterohepatic recycling are discussed in Sects. 6.2 and 6.5 for irinotecan and nonsteroidal anti-inflammatory drugs (NSAIDs), respectively. In both cases, gut bacterial enzymes remove the glucuronide moiety from the drug, which causes the drug to become active again and available to exert toxicities such as diarrhea and enteropathy. Variability in gut

Table 2 Indirect metabolism of drugs by gut bacteria associated with pharmacokinetic changes

Indirect metabolism						
Drug	Animal/human	In vivo/in vitro	Effect on PK (intact gut microbiome)	Enzyme	Diversity	Comments
Acetaminophen [35]	Animal	In vivo	↑ AUC and C_{max}	SULT1A1	N/A	P-cresol competes with acetaminophen binding to SULT1A1 → prevents host from detoxifying acetaminophen
Caffeine [33]	Human	In vivo	↓ CL	CYP1A2	↓ α ↑ β	Decreased CYP activity when treated with cefprozil
Metformin [36]	Animal	In vivo	↓ C_{max} and ↑ half-life	Oct1	N/A	Pharmacokinetic changes likely owing to ↓ Oct1 expression in the liver → altered hepatic uptake of metformin in vivo
Midazolam [33, 37]	Animal Human	In vitro In vivo	↓ C_{max}, AUC, and half-life four-fold ↓ CL	CYP3A; UGT	N/A ↓ α ↑ β	Low levels of CYP3A activity in GF mice decrease drug metabolism in vivo Decreased CYP activity when treated with cefprozil
Omeprazole [33]	Human	In vivo	↓ AUC metabolite ratio	CYP2C19	↓ α ↑ β	Decreased CYP activity when treated with cefprozil
Progestogens [38]	Human	In vivo	MPA had longest half-life	CYP450	N/A	Hydroxylation of progestins are likely CYP450 mediated
Triazolam [13, 39]	Animal	In vivo	Increased metabolite-to-parent drug ratio in SPF vs GF mice	CYP3A CYP3A11 CYP3A25	N/A	CYP activity higher for the livers of SPF mice (*Bacteroides and Escherichia coli*) Ciprofloxacin administration to SPF mice → significant ↓ mRNA expression of CYP3A11 in the liver

AUC area under the concentration–time curve, *CL* clearance, C_{max} maximum concentration, *CYP* cytochrome P450, *GF* germ-free, *mRNA* messenger RNA, *N/A* not available, *Oct1* organic cation transporter 1, *PK* pharmacokinetics, *SPF* specific pathogen-free, *SULT1A1* sulfotransferase 1A1, *UGT* uridine diphosphate glucuronosyltransferase (animal), ↑ increased, ↓ decreased

microbial β-glucuronidase activity, in UGT activity, and antibiotics that reduce β-glucuronidase activity may be factors in whether an individual develops diarrhea or enteropathy with these agents. As more data are developed in humans, giving a β-glucuronidase inhibitor, or a pre-biotic or probiotic, may soon be a reality in order to manipulate the gut microbiota to mitigate undesirable effects resulting from β-glucuronidase activity.

4 Volume of Distribution

The distribution of a drug within the body is affected by drug properties (e.g., lipophilicity, molecular size) and its interactions with body constituents, including binding to plasma proteins and tissues. The relationship between the apparent volume of distribution, drug binding, and anatomical volumes is given by:

$$\mathrm{Vd} = V_P + V_T\left(f_u / f_{uT}\right),$$

where V_P is the plasma volume, V_T is the tissue volume, and f_u and f_{uT} are the unbound fractions of drug in plasma and tissue, respectively. Additionally, although some drugs can passively distribute throughout body compartments, facilitated movement via transporters often governs distribution to and from various tissues. Transporters may also form physiological barriers such as the blood–brain barrier (BBB) and placental barrier and limit movement of drugs into tissues. Thus, microbiome effects on transporters, tissue binding, and plasma protein binding may alter the distribution of a drug within the body. These effects may have therapeutic consequences, for example, for a drug that must reach the brain to elicit a pharmacologic response.

Microbial-induced changes in plasma proteins have been reported, which could theoretically affect drug distribution. For example, gut bacteria may be related to serum albumin levels. In one study, higher abundance of *Sutterella*

was correlated with lower serum albumin levels in patients with colorectal cancer. However, the mechanistic basis underlying this finding is unclear [40]. Indirect relationships are also possible. To illustrate, phenolic fragments (e.g., hydroxybenzoic, hydroxyacetic and hydroxycinnamic acids, and hydroxybenzenes) are produced from flavonoids by bacterial microflora. Some of these metabolites form stable complexes with albumin in vitro [41] though appear to have limited potential to displace drugs from binding sites. Finally, several "uremic toxins" that are dependent on the presence of gut microflora, such as indoxyl sulfate, hippuric acid, and phenylacetic acid, are also highly bound in plasma to albumin [42, 43].

Relationships between the microbiome and the BBB have been identified. Germ-free mice show increased BBB permeability as compared with mice with a normal gut flora, with reduced expression of tight junction proteins persisting into adulthood [44]. Bacteria and bacterially released factors can reach the systemic circulation and affect immune cells to influence interactions with the BBB [45]. Other mechanisms that have been proposed include an indirect effect on cytokines, which then alters BBB transport sites and overall integrity [45].

5 Metabolism and Excretion

Determining the differential contributions of the intestine and liver to drug metabolism and excretion can be challenging and the contribution of the microbiome to these routes is emerging. It is increasingly evident that there is significant cross-talk between the intestine and liver and that bile acids, produced in the liver and modified by bacteria in the gut, are important signaling molecules that regulate host metabolism [46]. Bile acids achieve their signaling properties by binding to G-protein-coupled receptors such as the farnesoid X receptor and TGR5 [47]; and binding of bile acids to the farnesoid X receptor modulates CYP3A [48] and transporter activity [49, 50]. Other microbial products such as the secondary bile acid lithocholic acid (LCA), lipopolysaccharides produced from Gram-negative bacteria, and indole-3-propionic acid have also been shown to activate the nuclear receptor, PXR, another nuclear receptor involved in regulating drug metabolism and transport [51]. Animal data support the role of the gut microbiome in modifying host drug metabolism and transport. The protein expression of several CYPs and transporters such as Oatp and Bcrp1 were altered in germ-free and antibiotic-treated mice [52]; and ciprofloxacin-treated mice had significantly reduced LCA-producing bacteria in their feces. In germ-free mice given LCA, CYP3A expression was significantly elevated suggesting that LCA activated farnesoid X receptor and PXR [13]. Hepatic CYP3A and the activity of the CYP3A substrate midazolam were significantly lower in germ-free mice compared with conventional mice, suggesting that gut microbes may alter the metabolic activity of CYP3A [37].

One example of gut microbes altering host liver metabolism is with the analgesic acetaminophen (Table 2). Acetaminophen undergoes glucuronidation and bacterial glucuronidases can deconjugate the glucuronide metabolite allowing for reabsorption of the parent acetaminophen or further metabolism to sulfate and/or glucuronide conjugates. With antibiotic treatment, there is a decrease in the sulfate conjugate of acetaminophen [35]. In addition, gut bacteria produce a metabolite of aromatic amino acid metabolism, p-cresol, that competes with acetaminophen for binding to the enzyme sulfotransferase 1A1. Individuals who produce high levels of p-cresol were shown to have a low capacity for sulfonate acetaminophen [53]. Therefore, antibiotic therapy and/or high levels of the bacterially derived metabolite p-cresol could predispose individuals to the hepatotoxic effects of acetaminophen.

6 Drugs Affected by Microbiome Alterations with Clinical Significance

6.1 Digoxin

Digoxin is a cardiac glycoside for the treatment of atrial fibrillation and congestive heart failure [54]. Digoxin is a positive inotropic drug that inhibits the Na+/K+-ATPase pump, resulting in increased intracellular calcium in cardiac myocytes [55]. The narrow therapeutic window (target concentration range 0.5–2 mcg/L) of digoxin requires therapeutic drug monitoring [56]. Digoxin relative F is influenced by the formulation, with higher bioavailability in capsule formulations compared with tablets [57]. Digoxin F can also be influenced by malabsorption syndromes, gastrointestinal motility, and drug–drug or drug–food interactions [58]. Digoxin is a substrate for P-glycoprotein (P-gp) [59] and P-gp genetic polymorphisms impact digoxin pharmacokinetic (PK) variability as the partial area under the concentration–time curve (AUC) increased in subjects with the *MDR1* 3435TT genotype vs the *MDR1* 3435CC genotype ($p \leq 0.05$) [60]. Conflicting evidence exists and suggests no difference in digoxin partial AUC across *MDR1* 3435TT, CC, and CT genotypes [61, 62]. Epigenetic effects, via methylation of the *ABCB1* promoter region, also impact digoxin PK variability. Subjects with a high methylated epigenetic profile ($n = 15$) had higher digoxin partial AUC_{0-4} (5.12 ± 1.42 vs 4.31 ± 1.03 ng*h/mL, $p \leq 0.05$) and C_{max} (2.49 ± 0.18 vs 1.92 ± 0.26 ng/mL, $p \leq 0.05$) compared with subjects with a low methylated epigenetic profile ($n = 15$) [63].

Studies using various digoxin formulations provide evidence of the effect of *Eggerthella lenta* (*E. lenta,* previously named *Eubacterium lentum*) on urinary digoxin and digoxin reduction products (DRP) excretion. Digoxin absorption is proportional not only to exposure but to urinary digoxin excretion [58]. Urinary DRP excretion varies inversely with oral digoxin F [64, 65]. In healthy adults (n = 4) administered an oral tablet digoxin 0.25 mg once daily, DRP urinary excretion (described as a percentage of total digoxin and DRP excretion) was 45–80% compared to elixir and intravenous formulations that had lower DRP of 20–40%. Upon co-administration of a 5-day course of erythromycin or tetracycline antibiotics, DRP urinary concentrations and DRP excretion percentages dramatically reduced while digoxin serum concentrations increased [64]. In another study, healthy subjects (n = 22) received 0.4 mg of oral digoxin formulated as an encapsulated liquid or a tablet [65]. Mean cumulative digoxin urinary excretion was higher with the encapsulated liquid compared with the tablet (195 ± 8.6 vs 137.5 ± 6.3 mcg). In contrast, DRP urinary excretion (60.8 ± 5.5 vs 102.7 ± 9.5 mcg) and percentage DRP (23.5 ± 1.8% vs 41.2 ± 2.7%) was also lower with the encapsulated liquid compared with the tablet [65]. These results have been confirmed elsewhere [66].

Eggerthella lenta was identified as the bacteria that metabolized digoxin to an inactive reduced dihydrodigoxin metabolite in human gut flora [67, 68]. However, the presence as well as concentrations of *E. lenta* in the gut flora did not always correlate with DRP production [68]. Haiser et al. [19] later determined that a two-gene cytochrome-encoding operon (now referred to as cardiac glycoside reductase) was upregulated > 100-fold in the presence of digoxin in certain *E. lenta* strains.

Conditions that decrease and/or eliminate *E. lenta* activity may have clinical implications given the narrow therapeutic window of digoxin and target concentration range. Exposure to antibiotics during co-administration of digoxin is one example of a microbiome–drug interaction. Studies provide evidence of such an effect [64, 69] whereby eliminating *E. lenta* results in little to no urinary DRP formation. One would then expect increased digoxin F and increased systemic concentrations, which may impact the target concentration range. Other groups have speculated that diet may be clinically impactful given in vitro and in vivo animal studies support that *E. lenta* exposure to arginine decreased cardiac glycoside reductase operon expression and prevented the conversion of digoxin to dihydrodigoxin [19]. Monitoring of an individual's dietary protein intake may be needed during digoxin therapy as increased consumption of protein-rich foods, which contain arginine, would inhibit *E. lenta*-mediated digoxin reduction resulting in increased digoxin F.

6.2 Irinotecan

Irinotecan in combination with other agents is indicated for gastrointestinal carcinomas and small cell lung cancer. Irinotecan blocks DNA replication by inhibiting topoisomerase [70]. In hepatocytes, irinotecan is metabolized by carboxylesterase to SN-38, which is an active metabolite. SN-38 is then metabolized by UGT to form inactive SN-38G. Biliary excretion removes SN-38G into the intestinal lumen [70]. In Caucasian patients with cancer (n = 30), the UGT1A1*28 polymorphism and CYP3A4 phenotype (as measured by midazolam clearance) were statistically significant variables associated with irinotecan pharmacokinetics [71]. Midazolam clearance varied approximately four-fold during irinotecan therapy. Patients with the UGT1A1*28 polymorphism had higher SN-38 AUCs compared with patients without the UGT1A1*28 polymorphism [71].

Irinotecan-related toxicities such as neutropenia and diarrhea are dose limiting, potentially life threatening, and can be partially attributed to SN-38. *Escherichia coli* is a pathogen that produces β-glucuronidase, which converts SN-38G back to SN-38 in the intestinal lumen. Consequently, higher SN-38 intestinal lumen concentrations may increase the risk of diarrhea and localized enteric injury [72, 73] (Table 3). One animal study provided an initial glimpse of potential clinical implications. In this study, oral administration of a bacterial β-glucuronidase inhibitor protected mice from irinotecan-related toxicity [73], thus suggesting specificity of the β-glucuronidase inhibitor against bacterial, but not against mammalian-specific cells.

6.3 ICIs

Immune checkpoint inhibitors (ICIs) are indicated for a variety of solid tumor and hematological malignancies and induce an immune response by suppressing pathways involved in the negative regulation of the immune system. On the surface of T lymphocytes, cemiplimab, nivolumab, and pembrolizumab bind to programmed death receptor 1 (PD-1), while ipilimumab binds to the cytotoxic lymphocyte antigen 4 (CTLA-4) receptor. Atezolizumab, avelumab, and durvalumab target PD-1 ligands (PD-L1). Population PK analyses have identified intrinsic and extrinsic covariates having a modest effect on ICI PK variability. For most ICIs, statistically significant covariates on clearance include sex, body weight, estimated glomerular filtration rate, and immunogenicity [82–84]. Some have suggested that the modest influence of sex, renal function, and hepatic impairment on ICI clearance is due to various physiological mechanisms involved in clearance for monoclonal antibodies, specifically proteolytic catabolism in plasma and peripheral tissues and receptor-mediated endocytosis via target-mediated drug disposition [85, 86].

Table 3 Gut bacteria associated with pharmacodynamic effects of drugs

Drug	Animal/human	In vivo/in vitro	Organism or phyla	Effect on PD	Comments
Anti-PD-1 therapy [74]	Both	In vivo	*Faecalibacterium* and *Ruminococcaceae*	↑ Systemic and anti-tumor immunity	Enriched gut microbiome increased antigen presentation and improved effector T-cell function in the periphery and the tumor environment
Ipilimumab [75]	Both	In vivo	*B. fragilis* and/or *B. thetaiotaomicron* and *Burkholderiales*	↓ Tumor size	Antitumor effects of the CTLA-4 monoclonal antibody, ipilimumab depend on *Bacteroides* spp.
Irinotecan [27, 76]	Both	In vitro	*B. uniformis and Escherichia coli*	↑ GI toxicity	Variability of GI toxicity by anti-cancer drug, irinotecan, is a result of differences in types and levels of gut bacterial β-glucuronidases
NSAIDs [77, 78]	Both	In vivo	β-Glucoronidases	Enteropathy	Various gut microbes have β-glucuronidases that de-glucuronidate NSAIDs in the intestine causing increased susceptibility for enteropathy
Metformin [79, 80]	Both	In vivo	*E. coli* *Intestinibacter*	Regulation of glucose homeostasis	Metformin-treated microbiota transferred to germ-free mice improved glucose metabolism
Methotrexate [81]	Human	In vivo	Higher Firmicutes/Bacteroidetes ratio in patients with RA who were non-responsive to methotrexate	Response to methotrexate in patients with RA	Methotrexate-responsive patients had lower microbial diversity; methotrexate responders vs non-responders differed significantly in microbial gene abundance reflecting different microbial pathways

CTLA-4 cytotoxic lymphocyte antigen 4, *GI* gastrointestinal, *NSAID* non-steroidal anti-inflammatory drug, *PD* pharmacodynamics, *PD-1* programmed cell death receptor, *RA* rheumatoid arthritis, ↑ increased, ↓ decreased

Gut/intestinal microbiome composition affects ICI activity, efficacy, and toxicity. Intestinal recolonization of antibiotic-treated or germ-free mice with a combination of *Bacteriodes fragilis, B. thetaiotaomicron,* and *Burkholderia cepacia* restores the cytotoxic lymphocyte antigen 4-mediated anticancer response via induction of the interleukin-12-dependent, T-helper-1 immune response [87] (Table 3). In another mouse study, oral administration of *Bifidobacterium* species restored anti-PD-L1 antitumor activity resulting in dendritic cell maturation and increasing tumor-specific T-cell activity [88]. In humans, *Faecalibacterium* genus [89] and *Akkermansia muciniphila* [90] impact ICI activity and/or efficacy. In one study, patients with melanoma receiving anti-PD-1 immunotherapy ($n = 112$) were separated into responders (e.g., complete or partial response or stable disease for at least 6 months, $n = 30$) and non-responders (e.g., disease progression or stable disease less than 6 months, $n = 13$) [74]. An abundance of *Faecalibacterium* was observed in responders and was a strong predictor to anti-PD-L1 immunotherapy (hazard ratio = 2.92, 95% confidence interval 1.08–7.89). In another study, patients with metastatic melanoma treated with ipilimumab and whom had *Faecalibacterium* and other Firmicutes phylum composition ($n = 12$) had increased progression-free survival (PFS) and increased overall survival [89].

Immune checkpoint inhibitor activity and/or efficacy is also affected by microbiome diversity. Higher microbial fecal diversity was observed in patients with melanoma who responded to anti-PD-1 immunotherapy [74]. In other studies, antibiotic exposure resulting in a loss of microbial diversity (dysbiosis) decreased PFS and OS in patients with cancer receiving ICI immunotherapy [90, 91]. Derosa et al. [91] retrospectively analyzed patients with advanced renal cell carcinoma (RCC, $n = 121$) and patients with non-small-cell lung cancer ($n = 239$) receiving antibiotic therapy within 30 days (for oral) or 60 days (for intravenous administration) prior to starting PD-1/PD-L1 alone or in combination. An increased rate of primary progressive disease (75% vs 33%, $p < 0.01$) was observed in patients with RCC receiving antibiotic therapy ($n = 16$). Median PFS (1.9 vs 7.4 months, hazard ratio = 3.1, 95% confidence interval 1.4–6.9, $p < 0.05$) and median OS (17.3 vs 30.6 months, hazard ratio = 3.5, 95% confidence interval 1.1–10.8, $p < 0.05$) were also shorter compared with patients with RCC who were not receiving antibiotic therapy ($n = 105$). Progression-free survival and OS were also significantly shorter in patients with non-small-cell lung cancer receiving antibiotic therapy. The results of a shorter PFS and OS are consistent with another study in patients with non-small-cell lung cancer, RCC, and urothelial carcinoma receiving antibiotic therapy before or during PD-1/PD-L1 immunotherapy [90].

6.4 L-dopa

Levodopa (L-dopa) plus carbidopa is indicated to treat motor symptoms of Parkinson's disease such as tremors, stiffness, and gait. Levodopa is a prodrug requiring passage through the BBB and metabolism to dopamine by the human enzyme aromatic amino acid. Carbidopa is an aromatic amino acid inhibitor and is given in combination to reduce peripheral (non-central nervous system) metabolism of L-dopa to dopamine. Peripheral dopamine is unable to cross the BBB and is believed to mediate side effects [92]. Upon oral administration, there is known L-dopa serum concentration variability potentially impacting the pharmacodynamic and/or therapeutic response in patients with Parkinson's disease [93]. There is suggestion that *Helicobacter pylori* infection affects L-dopa F via duodenal mucosa disruption [94]. There is limited supportive evidence from studies that upon *H. pylori* eradication in patients with Parkinson's disease, L-dopa absorption increased and was associated with an improvement in motor symptoms [95, 96].

In 2019, a gut bacterial pathway involved in L-dopa metabolism was discovered [28, 29]. This pathway is distinct from the aforementioned host aromatic amino acid-mediated pathway of L-dopa metabolism. *Enterococcus faecalis* was identified as the strain that possessed a conserved tyrosine decarboxylase with the ability to metabolize L-dopa to dopamine. *Eggerthella lenta* was also identified as the strain mediating metabolism of dopamine to m-tyramine. In addition, carbidopa had little impact on the tyrosine decarboxylase-mediated *E. faecalis* pathway [28]. In October 2020, *Clostridium sporogenes* was identified in deaminating L-dopa in the gut whereby the 3-(3,4-dihydroxyphenol) propionic acid metabolite formed inhibits ileal motility in an ex vivo model. In addition, stool samples of patients with Parkinson's disease receiving L-dopa therapy contain this metabolite, thus suggesting active production by the gut microbiota [97]. The potential clinical impact is increased L-dopa dosage requirements in patients with Parkinson's disease given that several strains metabolize L-dopa prior to reaching the BBB for penetration into the brain [26].

6.5 NSAIDs

Nonsteroidal anti-inflammatory drugs are used to treat pain, inflammation, and fever. Non-steroidal anti-inflammatory drugs inhibit the enzyme cyclooxygenase, which is involved in the breakdown of arachidonic acid into prostaglandin, prostacyclin, and thromboxane. The majority of NSAIDs (e.g., aspirin, ibuprofen, indomethacin, and naproxen) are non-selective cyclooxygenase inhibitors, while celecoxib is a selective cyclooxygenase-2 inhibitor. Age, sex, and disease state contribute to NSAID PK variability [98–100]. In

addition, CYP2C9 genetic polymorphisms are also another contributory factor whereby meloxicam AUC was 2.4-fold higher in CYP2C9*1/*13 vs CYP2C9*1/*1 genotyped adults [100].

Non-steroidal anti-inflammatory drugs are glucuronidated in the liver and undergo enterohepatic circulation. In the intestine, NSAID-glucuronide conjugates are cleaved by microbiome-encoded β-glucuronidases resulting in reformation of NSAIDs in the parent form and increased aglycones. In theory, reformed NSAIDs and aglycones in the intestine contribute to mucosa damage and enteropathy. Evidence to support this mechanism is from a mice study, whereby β-glucuronidase inhibition protected against NSAID-induced enteropathy by indomethacin, ketoprofen, and/or diclofenac [77]. As discussed earlier with irinotecan, an area of potential clinical implication and/or development is to target bacterial β-glucuronidase inhibition to minimize NSAID-induced enteropathy. In addition, restoration of an altered intestinal mucosa caused by NSAIDs is another area of clinical intervention. Supplementation with probiotics such as several *Lactobacillus* strains, as well as use of a mucosal protective agent have shown decreased NSAID-associated small intestinal injury and inflammation in humans [78, 101–103].

7 What is the Future for Understanding Complex Drug–Microbe Interactions?

Modern and emerging chemical analysis methods, such as untargeted mass spectrometry-based metabolomics, are crucial tools necessary to untangle the complex influence of the gut microbiome on drug metabolism. Mass spectrometry is an ideal complement, revealing the chemical transformations and chemical changes associated with the gut microbiota, to genetic sequencing (e.g., next-generation sequencing such as 16S rRNA sequencing, shotgun metagenomic sequencing, microbial metatranscriptomics), which measures what microbes are present and measures alterations at the genetic level. Integration of these techniques will provide immensely different insights than currently possible.

One challenge, specific to mass spectrometry, is the amount of data which cannot be assigned a chemical name. While the annotation rate varies by experiment and sample type, it is not uncommon for the vast majority (> 90%) of all possible chemicals detected to go unannotated. The ability to detect and annotate parent drugs is strikingly good compared to other classes of chemicals as it is often relatively easy to purchase or acquire a genuine chemical standard. Conversely, drug metabolites are often poorly annotated, while generally amenable to detection, owing to the difficulty in acquiring genuine chemical standards. In silico methods to predict and identify metabolites (e.g., BioTransformer) [104] are likely to help overcome some of these aspects. Another data analysis solution is to utilize molecular networking [105], which aims to connect structurally related chemicals via similarity in mass spectrometry/mass spectrometry fragmentation. It is not uncommon for a parent drug to be connected to a desmethyl metabolite as well as other related unannotated compounds. A recently reported approach is to use a repository-scale data analysis as illustrated in Jarmusch et al. [106] in which clindamycin metabolites were connected via molecular networking of mass spectrometry/mass spectrometry data across multiple disparate metabolomics datasets. Regardless of the approaches, substantial progress in the ability to annotate chemicals in mass spectrometry data will enhance our understanding of microbial influence on drug metabolism. When elucidating interactions, it helps to have a chemical identification to connect with genetic sequencing information.

The most pronounced challenge is the difficulty in integrating microbiome and mass spectrometry (e.g., metabolomics) data in order to derive meaningful insights. Experimentally, one can explore the interactions by the quantitation of parent and known metabolites in multiple matrices, such as blood and feces, and model the contribution as reported by Zimmermann et al. [107] Computationally, there have been a few reported methods specifically intended to address this challenge that go beyond correlation measures such as Pearson, Spearman, and Kendall. Note, that correlation methods can be used to color molecular networks to enhance interpretation [108]. Microbe-metabolite vectors (mmvec), reported in Morton et al. [109] utilize the probability of metabolites and microbes co-occurring rather than a measure of correlation. The illustrative use reported in that paper connected *Pseudomonas aeruginosa*-associated molecules detected by mass spectrometry with taxa assignments from sequencing including *P. aeruginosa*. Certainly, one could imagine an analogous use in the study of the co-occurrence between drug metabolites and microbes. Last, visualization approaches, procrustes analysis [110], and molecular cartography [111, 112], will help link microbes to chemistry and inform their influences. In summary, one must carefully review the results and subsequent validation experiments should be the norm as comparisons between microbiome and metabolomics are bound to contain false discoveries.

8 Conclusions

The role of the gut microbiome and its clinical impact on pharmacokinetics and pharmacodynamics is still evolving. While there are examples of direct metabolism by gut microbes affecting drug pharmacokinetics, questions remain such as the specific species and strains involved, the redundancy and variability of the microbial community to metabolize these drugs, as well as other influences such as diet, other drugs, immunity, and circadian rhythms that may affect these activities. There is also evidence of indirect effects of the gut microbiome on drug metabolism involving gut microbial products such as bile acids interacting with host drug-metabolizing machinery. Further studies in humans to determine the clinical significance of these interactions as well as animal and/or in silico studies to investigate the mechanisms behind these effects are paramount. We have reviewed several examples of compounds in which alterations in gut microbial taxa can cause significant perturbations in pharmacokinetics and/or pharmacodynamics. The importance of assessing the role that the gut microbiome plays in the variability of xenobiotic metabolism and the resulting clinical effect in humans cannot be underestimated. As with other factors influencing individual variability, accounting for the influence of the microbiome is even more critical with narrow therapeutic range drugs. Currently, the available data on the gut microbiome's influence on drug pharmacokinetics and pharmacodynamics are not robust enough to translate to clinically actionable guidance. As the field advances, we anticipate that the gut microbiome's impact and the variability within and between individuals will be an important component in addition to genetics, diet, and other drugs in determining dose and response to drugs. Studying this highly variable and complex system will require a multi-pronged approach with animal, human, and systems biology models. There are immense challenges that remain in understanding the impact of the microbiome on drug metabolism; however, highly sensitive techniques such as mass spectrometry coupled with advanced in silico methods will certainly play a future role in revealing direct chemical transformation performed by microbes as well as the microbiota's indirect influences.

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