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HUMAN MICROBIOME

Human Gut Microbiota and Drug Metabolism

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Abstract

The efficacy of drugs widely varies in individuals, and the gut microbiota plays an important role in this variability. The commensal microbiota living in the human gut encodes several enzymes that chemically modify systemic and orally administered drugs, and such modifications can lead to activation, inactivation, toxification, altered stability, poor bioavailability, and rapid excretion. Our knowledge of the role of the human gut microbiome in therapeutic outcomes continues to evolve. Recent studies suggest the existence of complex interactions between microbial functions and therapeutic drugs across the human body. Therapeutic drugs or xenobiotics can influence the composition of the gut microbiome and the microbial encoded functions. Both these deviations can alter the chemical transformations of the drugs and hence treatment outcomes. In this review, we provide an overview of (i) the genetic ecology of microbially encoded functions linked with xenobiotic degradation; (ii) the effect of drugs on the composition and function of the gut microbiome; and (iii) the importance of the gut microbiota in drug metabolism.

Keywords Gut microbiome · Drug metabolism · Xenobiotics · Biotransformation · Genetic ecology

Introduction

The diverse and composite community of microorganisms residing in the human gastrointestinal (GI) tract is intensely entwined with human biology and is a key component of many processes like synthesizing vital nutrients and vitamins, digestion of complex polysaccharides, resistance against colonization of allochthonous microbiota, and immune maturation [1–3]. Although, the liver is considered as the predominant metabolic organ for biotransformation after dietary intake [4], however, recent studies indicate that the gut microbiota are the first to interact and metabolize/modify the chemical structure of numerous orally administered xenobiotics which include a wide variety of compounds ranging from environmental pollutants, dietary components, and therapeutic drugs. The dominant human gut bacterial phyla are mainly represented by Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia [5, 6]. However, the proportions of these phyla are documented to be sensitive to dietary habits, age, as well as disease conditions [7]. The gut microbiota contributes to approximately 3.3 million unique genes, which is roughly 150 times more than the human gene content [8]. This contributes to a large enzymatic repository in the gut, outnumbering that in the liver, which is capable of metabolizing numerous drugs and xenobiotics and manipulate their pharmacological effects either directly or indirectly, thus expanding the repertoire of metabolic reactions occurring within the human body [9, 10].

Although these modifications can have drastic consequences on health and treatment outcomes, our knowledge of specific gut bacterial strains and their genes and enzymes involved in xenobiotic metabolism is in infancy. An understanding of the mechanism of xenobiotic metabolism with respect to gut microbiota and hence intra-individual variations can help in planning for a better therapeutic regime as well as outcome. The microbial functions linked with

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xenobiotic metabolisms can be used as targets to modulate drug efficacy and also as diagnostic markers for clinical practices. In addition, such microbial genetic signatures can also help in developing precision medicine.

Here, we present an overview of our understanding of how the gut microbial consortia directly and indirectly modify the therapeutics and play an important role in health as well as disease. We focused on the current knowledge of the complex interactions between gut microbiota-derived functions and xenobiotics. We assessed the knowledge of overall genomic content and metabolic activities associated with the microbial community residing in the human gut. We also reviewed the current understanding of the chemical modifications of xenobiotic conjugates, drugs, and prodrugs accomplished through gut microbial consortia.

Overview of Xenobiotics and Its Metabolism

Xenobiotics can be defined as any substance that is foreign to the human body [11, 12]. These may be classified into two types: exogenous and endogenous. Exogenous xenobiotics are synthetic compounds that gain entry within the body through diet, therapeutic drugs, or inhaled as environmental pollutants. A few examples include drugs like antibiotics, antidiabetics, antiphychotics, food additives and artificial sweeteners like saccharine, antioxidants, and anti-inflammatory compounds, pollutants like pesticides, insecticides, cosmetics, and many more. Endogenous xenobiotics are synthesized within the body or are produced as metabolites from different biological processes. Though, they are indigenous to the body but have effects similar to exogenous xenobiotics including steroids, eicosanoids, bile acids, and certain fatty acids. The substances existing in unusually higher quantities are also termed as xenobiotics. The threat caused by these xenobiotics within human body or in environment includes carcinogenicity, toxicity, accumulation, and bioaccumulation. They can reside in fatty tissues for many years and lead to chronic problems, i.e., stunted growth, birth defects, learning disabilities, recurring diseases, brain function impairment, respiratory problems, cancer, and neurological, immunological, behavioral, and reproductive deficiencies [11, 13]. Human body has a natural detoxification mechanism to limit or diminish the acute or chronic toxicity of various xenobiotics (Fig. 1). Human gut and the liver have metabolic enzymes, which significantly reduce the concentration of any xenobiotic with certain chemical modifications before sending it to systemic circulation. This process is known as the "first pass effect." These chemical modifications or metabolic breakdown of xenobiotics occur in two phases, i.e., phase I and phase II metabolisms. Xenobiotics are chemically modified in phase I by introducing reactive functional groups via oxidation, reduction, hydrolysis, deamination, demethylation, dehalogenation, epoxidation, and/or peroxigenation. More often, phase I metabolism also makes the parent structure more polar after the end of these chemical transformations. Importantly, it is the phase II metabolism, after phase I, which significantly enhances

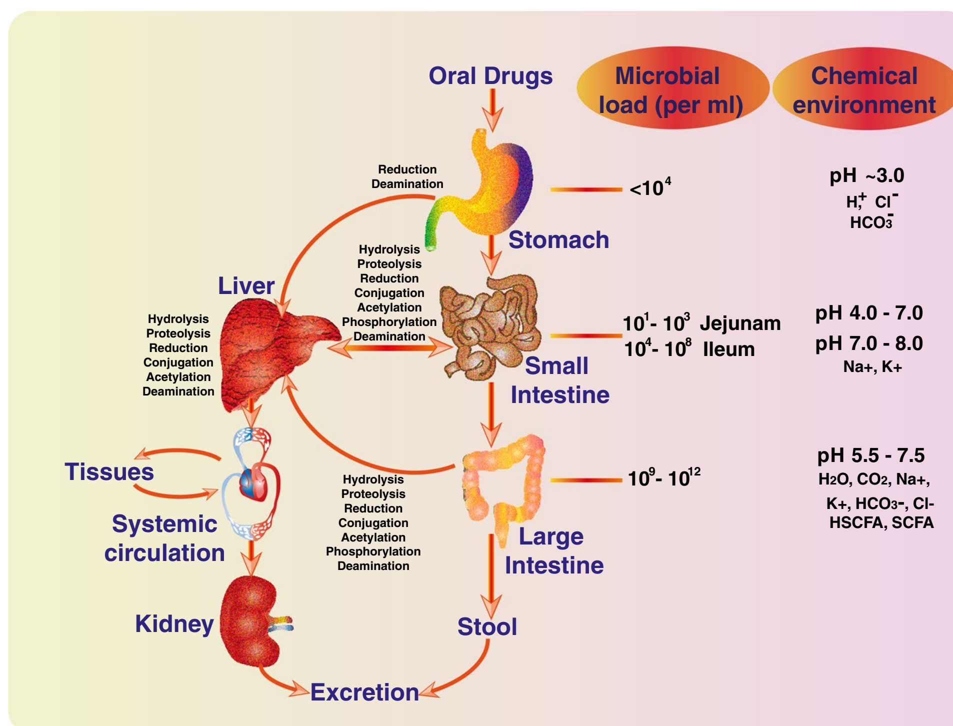

Fig. 1 Metabolism of orally administered drugs in the human gastrointestinal tract (GI) and other body sites. Microbial loads and drug modification functions are distinct in the different parts of gastrointestinal tract. Different parts of the GI tract have different microbial load and distinct chemical environments. The parent or modified drugs may reach to the liver through portal vein or directly excrete with feces depending on the absorption attribute of the compounds. Drug excretions also take place through urinary tract

hydrophilicity by conjugation of the phase I metabolite using glucuronic acid, sulfonic acid, glutathione, certain amino acids, or some other endogenous polar molecules. [14, 15]. The extreme acidic environment of the stomach (pH 1.5) also contributes to the chemical transformation of a broad range of pH-sensitive xenobiotics by modulating hydrolysis and reduction reactions in its scaffold. Such abiotic transformations are also important in xenobiotic metabolism in addition to direct involvement of microbial or host-encoded enzymes. Polymorphisms in xenobiotic-metabolizing genes affect an individual's response to pharmaceutical and dietary interventions [16]. Recent studies suggest the role of gut microbes in metabolizing therapeutics and convert them into wide range of metabolites. For most of the orally administered drugs, the major interplay with the microbiota occurs at the gastrointestinal tract. The diverse regions of gastrointestinal tract differ in nutrient content, epithelial cell physiology, pH (duodenum 6–6.5; jejunum and ileum 7–8; colon 5.5–7), and oxygen levels (small intestine: slightly aerobic; colon: strict anaerobic), thus providing diverse habitats to microbes and affecting the types of metabolic processes [17, 18]. The range of xenobiotics subject to gut microbial metabolism is wide and expanding. Several enzymes derived from the gut microbiota actively participate in the chemical transformation of dietary compounds, including complex polysaccharides, lipids, proteins, and phytochemicals. The modified metabolites serve as nutrients and are linked to a variety of health benefits as well as disease susceptibilities. Orally ingested xenobiotics can also be modified by the action of host-derived digestive enzymes in the oral cavity and digestive tract before they are absorbed into different tissues and systemic circulation [14]. The readily absorbed xenobiotics pass through gut epithelial cells, where host enzymes may further process them before transfer to the liver through the portal vein. After processing by the metabolic enzymes of the liver, xenobiotics and their metabolites infiltrate into circulatory system, reach different tissues, and affect the distal organs. These substances in the circulatory system are further metabolized and/or excreted into the gut lumen through bile duct or through kidneys into the urine (Fig. 1). The metabolites coming back into the intestinal lumen can either move into the large intestine, where they may be excreted out through feces, or be reabsorbed in the small intestine through enterohepatic circulation. The substances that are poorly absorbed within the small intestine enter into the large intestine where they may be transformed by the gut microbiota. The host cells can reabsorb the metabolites thus produced and enter into systemic circulation or interact with the epithelial cells lining the GI tract. Eventually, these metabolites are excreted either through urine or feces. Gut microbial metabolites can have altered bioavailability, bioactivity, and toxicity, regulate the expression and function of key liver enzymes and can interfere with the activities of human xenobiotic-metabolizing enzymes, altering the resulting metabolites from ingested substances [10]. The nutrient-rich, anaerobic intestine supports anaerobic bacterial colonization to metabolize indigestible substances like complex carbohydrates into short-chain fatty acids (SCFA) and regulate host metabolism while aiding digestion [17, 19].

Genomics and Genetics of Xenobiotic Metabolic Functions in the Gut Microbiome

The inter-individual variability in response to a treatment therapy can impose not only health hazards but cost implications also due to poor outcomes and prolong treatment durations. This variability is largely associated with the individual's gut microbiome structure and function. The increased diversity of enzymes and the distinct selection pressures shape the differential microbial xenobiotic biotransformation capacities. Often, the microbial biotransformation capabilities are different from that of host metabolism, shifting the pharmacokinetic and pharmacodynamic properties of xenobiotics and their associated metabolites. In many cases, the bacterial genes encoding for the enzymes mediating these reactions (Table 1) remain unidentified. The microbial community genome-encoded metabolic functions are highly conserved, based on the gut environmental conditions [19]. The gut microbiome tends to be more diverse in individuals with higher social interactions [20], thereby bringing more diversity of microbial enzymes and widening the range of metabolic processes occurring within the human body. Several researches have reported distinct inter-individual variability in these biotransformations [21–24]. The enzyme classes linked with xenobiotic metabolism like hydrolases, transferases, oxidoreductases, and lyases are extensively found within sequenced gut microorganisms. In a survey by Zimmermann and coworkers, a mechanistic understanding of microbiome-mediated drug metabolism has been provided. The ability of 76 intestinal bacteria to metabolize 271 oral drugs was measured, where approximately two-thirds of these screened drugs were metabolized by at least one bacterial strain [25]. By combining high-throughput genetics with mass spectrometry, they identified and validated 30 microbiome-encoded enzymes collectively converting 20 drugs to 59 metabolite candidates. They developed a gain-of-function approach by utilizing *Bacteroides thetaiotaomicron* capable of metabolizing 46 different drugs, to discover DNA fragments that confer drug-metabolizing capability in a heterologous host. They validated the metabolic activity of identified gene, *bt4096*, for diltiazem metabolism. Diltiazem is an oral calcium channel blocker and used to treat angina pectoris, arrhythmia, and hypertension. The intestinal levels of diltiazem and its metabolites

Table 1 Microbiota linked with chemical modification of drugs

Gut microbiota	Drug	Effect on drug	References
Helicobacter pylori	Levodopa	Impaired absorption and reduced efficacy	[140]
Eggerthella lenta possessing *cgr* operon	Digoxin	Drug inactivation	[141]
E. coli, *Enterobacter*, *Proteus* spp, *Klebsiella* spp, *Bacteroides* spp	Sulindac	Convertion into active compound sulindac sulfide	[142]
Cunninghamella	Flurbiprofen	Convertion into a variety of phase I and phase II metabolites, majorly 4′-hydroxyflurbiprofen	[143]
Clostridium and *Eubacterium* spps	Azo dyes and nitrated polycyclic aromatic hydrocarbons	Conversion into reduced azo and nitro compounds	[144]
Clostridium leptum	Nitrazepam	Nitro reduction to 7-amino-nitrazepam, a teratogenic metabolite	[145]
Escherichia	Metformin	Positive correlation with the hypoglycemic effect	[146]
Intestinibacter, *Clostridium* and *Romboutsia*	Metformin	Negative correlation with the hypoglycemic effect	[146]
E. coli DH5α	Thioguanine (TG) and mercaptopurine (MP)	Conversion into active metabolite thioguanine nucleotides (TGNs)	[147]

suggested that their deacetylation in the gut depends on *bt4096*. Using the same approach, they identified a total of 17 *B. thetaiotaomicron* gene products involved in metabolizing 18 different drugs to 41 different metabolites. These are *bt2068*, *bt2367*, *bt2961*, *bt3124*, *bt4091*, *bt1429*, *bt2366*, *bt0152*, *bt0217*, *bt0445*, *bt1192*, *bt0569*, *bt4096*, *bt4075*, *bt1006*, *bt1148*, and *bt3112*, confirming activity of each gene by targeted cloning and expression in *E. coli*. Thirteen drug-metabolizing gene products that collectively metabolize 16 drugs were identified from *Collinsella aerofaciens* and *Bacteroides dorei*. These include *ca00311*, *ca00815*, *ca01707*, *ca01846*, *ca02348*, *bd03934*, *bd03988*, *bd00571*, *bd00665*, *bd03379*, *bd03642*, *bd03937*, and *bd03091*. The genomic presence of homologs of these identified drug-metabolizing gene products was assessed by enrichment analysis for the drug-metabolizing activities across the 76 bacterial strains. Many identified gene products demonstrated significant enrichment and suggested their contribution in the observed bacterial drug metabolism. An in silico analysis by Sharma and coworkers predicted the gut bacteria and the metabolic enzymes by developing DrugBug database, an in silico tool that anticipates biotransformation of drugs through microbial enzymes mostly produced by the microbiota residing in the GI tract. This metabolic enzymes database was developed from genome of 491 human intestinal bacteria, which carried 324,697 metabolic enzymes allotted with EC numbers. From the six EC classes, the highest (65.75%) number of molecules were metabolized by enzymes belonging to EC1 and EC2 classes, i.e., oxidoreductases and transferases, respectively, whereas only 6.83% of the total molecules were metabolized by enzymes from EC5 and EC6 classes, i.e., isomerases and ligases, respectively. The group has validated the DrugBug tool by predicting metabolic enzymes for digoxin, a cardiotonic glycoside. The molecular structure of digoxin, consisting of three sugar moieties and one aglycone digoxigenin moiety, suggested three possible sites for metabolic reactions: 3β-OH group, C-17 attached lactone ring, and the sugar moieties. The three potential enzyme classes to metabolize digoxin were predicted to be oxidoreductase (EC1) for the reduction of lactone ring, transferase (EC2) for the addition of sugar moieties, and hydrolases (EC3) for the removal of sugar moieties. Using DrugBug, the enzymes identified were glucosyltransferases and mannosyltransferase from the genus *Citrobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, and *Enterococcus* and FAD-dependent oxidoreductases, disulfide reductase, and hydroxylases from the bacterial genus *Escherichia*, *Providencia*, *Klebsiella*, *Eggerthella*, and *Streptomyces*. The ten drugs used in the study are ginsenoside Rb1, quercetin-3-glucoside, loperamide oxide, methamphetamine, omeprazole, sorivudine, lactulose, zonisamide, cycasin, and cyadox [26]. Recent studies have also suggested age-related variations in gut microbial composition. In a study with 69 people, including centenarians (age 99–104) and semi-supercentenarians (age 105–109), the number of reads for genes involved in xenobiotic transformation and metabolism were higher with a concurrent decrease in genes devoted to carbohydrate metabolism with the progression of age. These functional shifts were even more prominent in the microbiome of centenarians and semi-supercentenarians. The pathways for pentose phosphate (ko00030), starch and sucrose (KEGG pathway no. ko00500), and amino sugar and nucleotide sugar (ko00520) metabolism were less expressed whereas ethylbenzene (ko00642), caprolactam (ko00930), toluene (ko00623), and chlorocyclohexane and chlorobenzene (ko00361)

degradation pathways were more pronounced. The group of young individuals had higher levels of genes for glycerophospholipid (ko00564) and sphingolipid (ko00600) metabolism, whereas, centenarians and semi-supercentenarians showed more reads for glycerolipid (ko00561) and alpha-linoleic acid (KEGG pathway no. ko00592) metabolism. As age progressed, there was a progressive increase in genes for threonine (ko00260), tyrosine (ko00350), tryptophan (ko00380), serine, and glycine amino acid metabolism. The genes for lipopolysaccharide biosynthesis (ko00540) were also more pronounced in aged people, which can be related with the existence of pathobionts and the low levels of chronic inflammation [27]. Another metagenomic study by Almeida and coworkers, retrieving 13,133 human gut metagenomic datasets suggested that the clades of the known gut colonizers still contain considerable uncultured diversity. In their study, Coriobacteriaceae (20.6%), Ruminococcaceae (9.9%), and Peptostreptococcaceae (7.4%) were the three most frequent families; *Collinsella* (17.7%), *Clostridium* (7.3%), and *Prevotella* (4.4%) were the top genera found. Screening for the presence of secondary metabolite biosynthetic gene clusters (BGCs) within both the human gut reference (HGR) and unclassified metagenomic species (UMGS) was done using antiSMASH. Over 200 BGCs coding for sactipeptides, nonribosomal peptide synthetases (NRPSs), and bacteriocins were detected. Between the UMGS and HGR genomes, KEGG pathways involved in carbohydrate metabolism had differential abundance, representing discrete metabolic resemblance between the cultured and uncultured species. UMGS had less abundant genes for antioxidant and redox functions, which signified lower tolerance to reactive oxygen species. Also, among the UMGS genomes, genes encoding for iron–sulfur and ion binding were found enriched suggesting the UMGS may be better adapted to niches with low oxygen tension or high iron concentration, since such conditions generate high levels of ferrous ions in their environment [28].

Effects of Drugs on the Composition and Functions of Gut Microbiome

Gut microbial perturbations due to diet, drugs, alcohol, and other environmental factors can initiate and induce the progression of metabolic disorders [29, 30]. Several health disorders are associated with lower gut microbial diversity and reduce functional potency of microbial genomes when compared to healthy subjects [31, 32]. The differential inter-individual response to therapeutic drugs depends on how the gut microbiome functions at the time and on the potency of perturbation (Table 2). The early and long-term treatment outcomes are influenced by the proliferation of either the pathogenic, beneficial, or resistant microbiota after termination of drug therapy. Recent studies suggest that prolonged drug therapy during tuberculosis treatment leads to remarkable depletion of numerous immunologically significant commensal gut bacteria, and this dysbiosis persists at least for 1.2 years [33, 34]. Similarly, another study for a short course of 4 days with a cocktail of gentamicin, vancomycin, and meropenem in

Table 2 Different drugs linked with gut microbial dysbiosis

Drug	Type of drug	Effect on gut microbiota	References
Metformin	Anti-diabetic drug	Increased SCFA-producing gut bacteria such as *Butyrivibrio*, *Bifidobacterium bifidum*, and *Megasphaera*	[148]
Metformin	Anti-diabetic drug	Increased abundance of mucin-degrading gut bacteria *Akkermansia muciniphila*	[148]
Metformin	Anti-diabetic drug	Decreased abundance of *Bacteroides fragilis*	[149]
Metformin	Anti-diabetic drug	Increased abundance of *Escherichia* species	[148]
Metformin	Anti-diabetic drug	Significant decrease in microbial richness	[43]
Metformin	Anti-diabetic drug	Reduced abundance of *Faecalibacterium* in Indian and Danish subjects	[43]
Vildagliptin	Anti-diabetic drug	Reduced abundance of *Oscillibacter* species and increased the abundance of *Lactobacillus* species	[150]
Dapagliflozin	Anti-diabetic drug	Reduced Firmicutes: bacteriodetes ratio	[151]
Indomethacin	Non-steroidal anti-inflammatory drug (NSAID)	Increased Firmicutes: bacteriodetes ratio	[152]
Aspirin	NSAID	Shift in gut microbial composition regarding *Prevotella*, *Bacteroides*, Ruminococcaceae, and *Barnesiella*	[153]
Celecoxib and Ibuprofen	NSAID	Increased abundance of Acidaminococcaceae and Enterobacteriaceae	[153]
Ibuprofen	NSAID	Enrichment in Propionibacteriaceae, Pseudomonadaceae, Puniceicoccaceae, and Rikenellaceae species	[153]

healthy subjects, suggested that the gut microbiota was restored to near-baseline composition within 1.5 months. This treatment resulted in the depletion of *Bifidobacterium* species, *Methanobrevibacter smithii*, and butyrate producers while enterobacteria and other pathobionts, such as *Fusobacterium nucleatum* and *Enterococcus faecalis*, were thriving. However, nine species common in all the study subjects before treatment initiation were not recovered in most of the subjects even after 6 months [35]. Both categories of therapeutic drugs, i.e., antibiotics and non-antibiotic drugs, can induce the acquisition of antimicrobial resistance (AMR) [36] and aid in intra/inter-species transmission of AMR genes within the gut microbiota, causing the emergence of potential drug-resistant pathogens. Paracetamol and opioids have a positive association with Streptococcaceae. Selective serotonin reuptake inhibitors (SSRIs) are negatively associated with Turicibacteraceae abundance but positively associated with *Eubacterium ramulus* and *Bifidobacterium dentium*, which are abundant in PPI users. The abundance of *Streptococcus salivarius* increases with the intake of oral steroids, PPIs, platelet aggregation inhibitors, vitamin D supplements, and SSRI antidepressants. Laxatives increase the abundance of *Bacteroides* and *Alistipes* and *Clostridium leptum* [37]. IBD patients' guts have lower abundances of beneficial microorganisms, including butyrate-producing *Faecalibacterium prausnitzii* and *Prevotella copri* [38]. The use of oral steroids like levothyroxine increases the abundance of *Methanobrevibacter smithii* and Actinomyces [39]. A study suggested that the gut microbiota composition of drug-sensitive epilepsy was similar to healthy control. However, the gut microbiota of drug-resistant epilepsy was altered to an increased abundance of microbes primarily from the phylum Firmicutes, with a reduction in commensal gut bacteria (Table 2). Also, there was a significant increase in the phylum Verrucomicrobia in these patients [40]. Studies also suggest that drug usage also modifies the functional component of the gut microbiota. A widely used antidepressant, duloxetine, induces higher diversity in synthetic bacterial communities and affects the purine metabolism of *B. uniformis* and *C. saccharolyticum* [41]. A recent study by Vila and coworkers has shown a correlation between 411 microbial pathways and the usage of 11 drugs. Proton-pump inhibitors (PPI), metformin, and laxatives were found to have a strong association with the abundance of microbial taxonomies and pathways. The largest associations were found for PPIs with approximately 40 altered taxa and 166 altered microbial pathways. Altered functions included the increase of fermentation; NAD metabolism; fatty acid and lipid biosynthesis; purine deoxyribonucleoside degradation; and biosynthesis of L-arginine. Metformin caused changes in the gut microbiome's metabolic potential, including increased quinone biosynthesis, butanoate production, polymyxin resistance, and sugar derivative degradation pathways [42, 43]. Another study suggested that 27% of non-antibiotic human-targeted drugs repressed the growth of at least one gut bacterial species [36]. Around 47 drugs, which were anti-infectives against viruses or eukaryotes, exhibited anti-commensal activity [36]. Artificial sweeteners can also affect the gut microbiome positively or negatively, leading to altered metabolic changes impacting SCFA production, insulin sensitivity, inflammation, or lipid metabolism [44]. In a recent in vitro study, the effects of eight oral antiviral drugs, which include lopinavir, chloroquine, ritonavir, darunavir, arbidol, ribavirin, oseltamivir, and favipiravir, were studied on the metabolism of four glycosides (i.e., geniposide, polydatin, glycyrrhizin, quercitrin) and on the activities of three major glycosidases (β-glucosidase, β-glucuronidase, α-rhamnosidase) from gut microbiota (Table 1) and determined by LC–MS/MS [45]. One or more anti-coronavirus drugs at 100 μM concentration extensively inhibited the metabolism of all four glycosides. Chloroquine and darunavir had no inhibition effect on α-rhamnosidase, while they had a weak effect on β-glucosidase and β-glucuronidase, respectively [45].

Microbial Functions and Drug Metabolism

Many recent studies suggest that the gut microbiota manipulates xenobiotic metabolism through direct and indirect mechanisms and alters the effectiveness, quality, and toxicity of xenobiotics and drugs [46]. The two broad mechanisms by which gut microbiota mediates drug metabolism: direct biotransformation mechanism where metabolism of drugs is performed by microbial enzymes, while indirect biotransformation mechanism suggests the impact of microbial metabolites on host receptors and signaling pathways. The gut microbiota can directly influence a person's response to a specific drug through directly interacting or by producing enzymes and inducing major or minor biochemical transformations in the drug to make it either more or less active/inactive or produce toxic metabolites [47–50] (Table 1). Besides this, microbiota indirectly interact with administered drugs by inducing reactivation of secreted inactive drug metabolites [51], producing metabolites to compete with drugs for the same host-metabolizing enzymes [52], modulating immune cell dynamics during immunomodulatory interventions like conditioning [53], and altering the levels of metabolizing enzymes in the intestine and liver [54–56]. As the gut microenvironment is anaerobic or rarely oxygenated, the drug metabolism by gut microbiota majorly involves hydrolytic and reductive biotransformations [57, 58].

Direct Biotransformation of Drugs by Microbial Enzymes

Direct microbial biotransformation of ingested xenobiotics takes place when the compounds reach the intestinal lumen where a collection of bacterial enzymes capable of performing various chemical reactions acts upon them. The abundant glucuronides in the human intestine are processed by bacterial-glucuronidase enzymes to produce glucuronic acids from conjugated compounds [59]. According to one study, there are 3013 microbiome-encoded-glucuronidases with distinct functional capabilities for different glucuronide substrates [60]. Besides the hydrolytic biotransformations of the glucuronide conjugates, there are other abundant intestinal bacterial enzymes (Table 1) like polysaccharide lyases, transferases, reductases (nitroreducases and azoreductases), lipases, mono and dioxygenases, sulfatases, endoglycosidases, and glycyl radical enzymes [10, 61]. The administration of probiotics has been shown to alter the structural and functional (enzyme activity) components of the intestinal microbiota. A study by Kim and coworkers suggested that the coadministration of probiotic strains with drugs resulted in altered pharmacokinetics of the drugs. The introduction of *Lactobacillus reuteri* K8 resulted in an increased abundance of Bifidobacteria, Enterococci, Clostridia, and Cyanobacteria. It enhanced the degradation of acetaminophen by itself and by the gut microbiota and arylsulfate sulfotransferase activities without changing the intestinal metabolic activities. While *Lactobacillus rhamnosus* K9 administration increased Deferribacteres and decreased Bifidobacteria, it had no effect on acetaminophen degradation [62, 63]. According to one study, NSAID-associated GI-toxicity is induced by glucuronidase enzyme (encoded by the gus gene)-mediated aglycone release, which then initiates a cascade of events resulting in cell injury when it comes into contact with enterocytes [64]. However, inhibition of bacterial-glucuronidase by Inh-1 [1-((6,8-dimethyl-2-oxo-1,2-dihydroquinolin-3-yl)-3-(4-ethoxyphenyl)-1-(2-hydroxyethyl) thiourea] protected mice from NSAID-induced enteropathy caused by indomethacin, ketoprofen, and diclofenac [65]. For the cisplatin-associated hepatotoxicity, the gut microbial role remained unclear. However, when antibiotics were co-administered with cisplatin, it resulted in reduced toxicity, thereby confirming the gut microbiota regulated cisplatin-induced hepatotoxicity [66].

Microbial Regulation of Hepatic Enzyme Functions and Drug Metabolism

Research with germ-free animals revealed that gut bacteria play a significant role in regulating functions of host-encoded metabolic enzymes. Cytochrome P450s, enzymes encompassing heme as cofactor and a major subset is phase-I oxidation enzymes, have an important role in drug metabolism and biosynthesis of endogenous biomolecules. P450s have hepatic as well as extrahepatic expression. The human CYP3A4 is involved in metabolizing more than 60% of all drugs [67]. The mRNA and protein expression of its mouse homolog CYP3a11 were significantly lower in the livers of adult germ-free mice [67]. However, the same study also suggested enhanced gene expression of Cyp1a2 (metabolic deactivation of certain anticancer drugs and activation of procarcinogens) and Cyp4a14 (metabolism of eicosanoids and fatty acids) in germ-free mice. The host-associated xenobiotic metabolizing enzymes (XMEs) involved in phase II metabolism were also differentially expressed in germ-free rats compared to conventional rats [54].

Hydrolysis of Drugs, Prodrugs, and Xenobiotic Conjugates

Hydrolases are the enzymes produced by both the host and gut microbiota to break down orally ingested substances into smaller molecules that may be further metabolized. These enzymes add a water molecule to a substrate, which is followed by the bond cleavage. The hydrolases frequently found in the gastrointestinal tract are proteases, sulfatases, glycosidases, and esterases. The proteases act on the peptide bonds, chopping off the amino acid linkages in the polypeptide chain. They are classified into four classes on the basis of their catalytic sites: cysteine proteases, serine proteases, aspartic proteases, and metalloproteases. Different regions of the human intestine are equipped with different types of proteases. The human colon is dominated by many microbial cysteine and metalloproteases, whereas the pancreatic serine proteases are majorly present in small intestine. These enzymes differ in substrate specificities leading to potentially different therapeutic outcomes [68, 69]. The study of ~ 10 million genes from human gut metagenomic catalog resulted in recognizing 285 putative serine protease sequences from human intestinal microbiota. Fifty-six genera from five different phyla, namely Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Fusobacteria contributed to these sequences [70]. Single amino acid prodrugs or

peptide prodrugs form the simplest group of drug-conjugates containing a short linker adhered to the drug [71]. They have negligible activity in the conjugated state until the drug is released by the target protease to gain high potency. Legumain, a cysteine protease, recognizes asparagine (Asn) to a much higher extent to aspartate (Asp) at the P1 position of very high specificity [72]. Thus, many legumains target prodrugs contain Asn conjugated with cytotoxic drug [73]. The hydrolysis of carbohydrate/glycosyl conjugates is carried by glycosidases. These enzymes hydrolyze glycosidic bonds using a dyad of carboxylic acid residues and a water molecule, releasing free sugars [74]. The three major glycosidases are α-rhamnosidase, β-glucosidase, and β-glucuronidase. These are largely disseminated across gut microbiota [75, 76]. The most important enzyme for xenobiotic metabolism is β-glucuronidase, which hydrolyzes glucuronides which enter the gut through biliary secretion. A good example of this mechanism is provided by the chemotherapeutic drug irinotecan, which is metabolically inactivated via glucuronidation in the liver. However, upon entering the intestine, it is metabolically reactivated by the activity of bacterially expressed β-glucuronidase [77]. This reactivation causes severe, dose-limiting GI toxicity [78–81]. Sulfatases, the hydrolytic metalloenzymes, hydrolyze sulfate esters generated by phase II metabolism using the unusual amino acid residue formyl glycine on their active site [82–84]. The hydrate form of this residue is thought to undergo trans-esterification with a sulfate ester substrate to generate a tetrahedral intermediate that breaks down to release sulfate and reform the aldehyde [85]. The microbial sulfatases reactivate sulfated metabolite conjugates, which enter the GI tract for excretion [86–88]. This reactivation enables these molecules to either undergo enterohepatic recirculation or exert local effects on gut epithelium. Eighty percent of gut microbial sulfatases arise from Bacteroidetes and are Ser-type enzymes, whereas the remaining 20% are Cys-type sulfatases arising from Firmicutes, Proteobacteria, Fusobacteria, Verrucomicrobia, and Spirochaetes [89]. Using protein structure-guided methods, Ervin and coworkers have identified 728 discrete microbiome-encoded sulfatase proteins from 4.8 million unique proteins in the HMP stool sample database. A total of 1766 gut microbial sulfatases were identified from the 9.9 million sequences present in the integrated gene catalogue database [90]. Hydrolytic reactions alter both the physical properties and activities of xenobiotics and their metabolites. For example, removal of a glucuronide in the gut lumen is generally accompanied by a decrease in polarity that can allow reabsorption by host cells and thereby extend the lifetime of a molecule within the body, as seen with glucuronide conjugates of nonsteroidal anti-inflammatory drugs and the cancer therapy irinotecan [51, 65]. Esterases belong to hydrolases class of enzymes, which catalyze the conversion of an ester group to a carboxylic acid and an alcohol by a hydrolysis reaction. These include carboxylesterases, acetylesterases, and arylesterases. *Rhodobacteria sphaeoides*, *Bacillus subtilis*, *Geobacillus stereothermophillus*, *Pseudmonas fluorescens*, *Arthrobacter globiformis*, *Archaeoglobus fulgidus*, *Alicyclobacillus acidocaldarius*, *Lactobacillus casae*, *Klebsiella* sp., *Sulfolobus solfataricus*, *Serratia* sp. SES-01, and *Sulfolobus tokodaii* exhibited carboxylic ester hydrolase activities to hydrolyze phenylacetate, 4-nitrophenylacetate, naphthylbutyrate, and some drugs such as ketoprofen, aspirin, and naproxen [91]. Enzymes like arylsulfatases and β-glucuronidases produced by gut bacterial flora can cleave the conjugates formed by the sulfo- and glucuronosyltransferases, leading back to the parent compound, thereby enhancing the half-life of the drug [92]. Often, hydrolysis is a prerequisite before further processing, for instance, the fermentation of sugars released from indigestible polysaccharides [93]. The end products of these reactions frequently support growth of gut commensals and their survival within gut [10].

Modifications of Drug Scaffold by Reduction

Reductive transformations are performed by enzymes and reductases, by making use of a variety of cofactors like flavin, NAD(P)H, i.e., NADH or NADPH, molybdenum cofactor, Fe–S clusters, (siro) heme, and other metallocofactors. With these enzymes, gut microbes can reduce various functional groups by transferring hydride equivalents or electrons (H^+, $2e^-$) to substrates [94–96]. The reductive transformation usually leads to decrease in polarity of metabolites by modifying the charge, electrophilicity, or hybridization. This usually influences the activity and life span of these metabolites in the body [97–99]. The six major reductive transformations include nitro (NO_2), azo (–N = N–), ketone (–C = O), alkene (–C = C–), sulfoxide (–S = O), and N-oxide (–N–O) [100]. The bacterial genera *Clostridium* sp., *Eubacterium* sp., *Clostridium leptum*, *C. paraputrificum*, *C. perfringens*, and *C. clostridiiforme* exhibited nitroreductase and azoreductase activities in the human GI tract [101–104]. Misal and Gawai have classified azoreductase superfamily into five different groups, depending upon their preference for nicotinamide and flavin: (1) flavin-containing NADPH-dependent azoreductase [105, 106], (2) flavin-containing NAD(P)H-dependent azoreductase [107–110], (3) flavin-containing NADH-dependent azoreductase [111, 112], (4) flavin-containing NAD(P)H-dependent quinine oxidoreductases [113, 114], and (5) flavin-free NAD(P)H-dependent azoreductase [115–118]. Azo drugs like olsalazine, asacol, pentasa, balsalazide, azulfi

dine, and salazopyrin for the treatment of ulcerative colitis and inflammatory bowel disease (IBD) [119, 120] are prodrugs, having 5-aminosalicylate (5-ASA), a non-steroidal anti-inflammatory molecule, and an inert carrier are linked with an azo bond, in order to avoid their rapid adsorption [121, 122]. The activities of these drugs rely upon azo bond cleavage by azoreductases secreted by gut microbiota, to release 5-ASA [100]. The enzymes nitroreductases catalyze the reduction of nitro-containing molecules. The bacterial nitroreductases can either be oxygen-insensitive (type I) or oxygen-sensitive (type II) or both [123, 124]. On the basis of electron donor preference, the well-studied oxygen-insensitive nitroreductases are broadly classified into major protein group for employing nicotinamide adenine dinucleotide phosphate (NADPH) and minor protein group employing both, nicotinamide adenine dinucleotide (NAPH) and NADPH as electron donors [125]. Chloramphenicol, a natural nitroaromatic compound, is reduced to the amine metabolite through nitroreductases exhibited by *Bacteroides fragilis*. The amine metabolite displays very weak antibacterial activity [126, 127].

Modifications of Drug by Adding and Removing Functional Groups

The gut microbiota also performs a wide range of biotransformation reactions involving transfer of functional groups (Fig. 2). These reactions include deamination, demethylation, deacylation, decarboxylation, dehydroxylation, and oxidation. Transferase enzymes that move the functional groups amid two substrates through nucleophilic substitution reactions execute this activity. The action of gut microbiota-encoded deaminases in the conversion of 5-fluorocytosine to 5-fluorouracil is responsible, at least in part, for toxicity associated with 5-fluorocytosine chemotherapy [58, 128]. Using an anaerobic in vitro study, the biotransformation of 12 structurally diverse polymethoxyflavones (PMFs) was reported by gut bacteria *Blautia* sp. MRG-PMF1 to identify the metabolic intermediates. 5,7-dimethoxyflavone, 5-hydroxy-7-methoxyflavone, 3,5,7-trimethoxyflavone, 5-hydroxy-3,7-dimethoxyflavone, 5,7,4′-trimethoxyflavone, 5-hydroxy-7,4′-dimethoxyflavone, 3,5,7,4′-tetramethoxyflavone, 5-hydroxy-3,7,4′-trimethoxyflavone, 5,7,3′,4′-tetramethoxyflavone, 3,5,7,3′,4′-pentamethoxyflavone, 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, and 5,3′-dihydroxy-3,7,4′-trimethoxyflavone were converted to chrysin, apigenin, galanga, kaempferol, luteolin, and quercetin after complete demethylation [129]. Additional reactions usually require chemically activated cosubstrates like acetyl

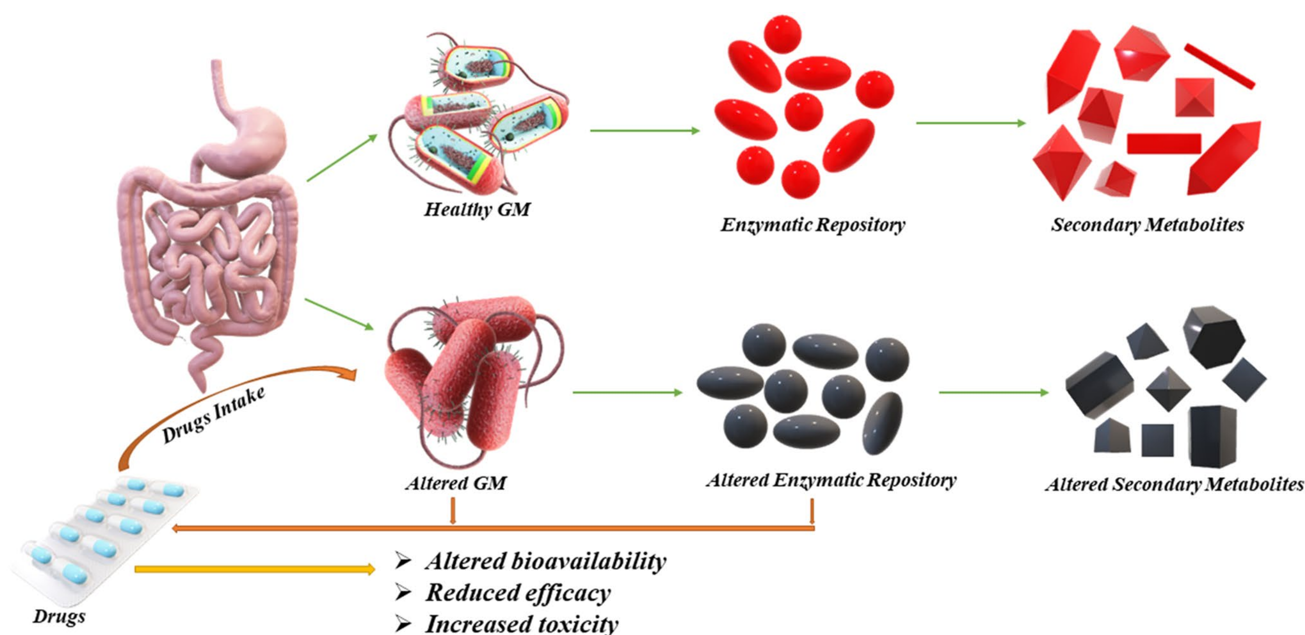

Fig. 2 Representation of bidirectional effect of gut microbiota and drugs. In a healthy individual, the gut microbiota (GM)-encoded enzymes synthesize and modify metabolites, which help in maintaining good health. However, during therapeutic interventions, there is an alteration of compositions and/or functions of GM, which act on drugs differentially, thereby resulting into altered bioavailability, reduced efficacy, and increased toxicity of drugs

coenzyme A (CoA), S-adenosylmethionine (SAM), or adenosine triphosphate (ATP). The enzymatic removal of acyl groups usually depends on hydrolysis. Demethylases utilize cofactors like tetrahydrofolate and cob(I)alamin proficient in nucleophilic catalysis [130, 131].

Other Microbial Enzymes Involved in Biotransformations Within the Gut

Lyases

Lyases are the enzymes which catalyze the C–C or C–X (X may be N, O, P, S, or halides) bond breakage. These enzymes catalyze the reactions without requiring oxidation or addition of water. Polysaccharide lyases (PLs), along with glycoside hydrolases, encoded by the genome of intestinal microbiota, help in utilizing dietary or mucin glycanss [18, 132]. The host-mediated xenobiotic metabolism is usually performed by cytochrome (CYP) P450 in the liver. During detoxification process, the liver produces glutathione conjugates, which are then carried with the bile to intestine where the gut bacteria-encoded C-S lyases act upon them. Alginate (sodium alginate ref. no. 00148), used as stabilizing and gelling agents in food and pharmaceuticals [133], is a linear polysaccharide, consisting of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G), was thought to be non-digestible by humans. However intestinal bacteria like *Bacteroides thetaiotaomicron*, *B. xylanisolvens*, and *B. ovatus*, isolated from human stools can utilize alginate in vitro to form healthy short-chain fatty acids [134, 135]. Bacteria like *Bacteroidetes*, *Lactobacilli*, and *Bifidobacteria* have positive association with alginate presence in the gut of rats [136]. In a recent study, a polysaccharide lyase family 6 alginate lyase (*Bcel*PL6) was identified in the genome of gut bacteria *Bacteroides cellulosilyticus* [137]. Another study reported a gene (BT_3328) encoding a novel polysaccharide lyase, BtCDH from *B. thetaiotaomicron*, capable of degrading the dermatan sulfate, chondroitin sulfate, and hyaluronic acid of glycosaminoglycans, an important player in host–microbial interactions [138].

Radical Chemistry

Radical biotransformations are useful in catalyzing those reactions, which may be difficult to catalyze by polar or other modes of biotransformation. The major gut bacterial enzymes involved in radical mediated biotransformations are glycyl radical enzymes (GREs), cobalamin (B12)-dependent enzymes, and *S*-Adenosylmethionine (SAM) enzymes [10]. These enzymes produce high-energy intermediates having an unpaired electron, and these reactions are usually sensitive towards oxygen and require significant amount of energy. These enzymes are highly abundant in the intestinal microbiota and often execute primary metabolism in anaerobic bacteria, thereby influencing the fate of xenobiotics directly or indirectly within the human body. The SAM enzymes are involved in drug resistance are Cfr and its homolog RlmN, which methylate adenine base A2503 located in the peptidyl transferase center of 23S rRNA (*E. coli* numbering), at C8 and C2 positions, respectively. This modification develops resistance towards ribosomal antibiotics like pleuromutilins, oxazolidinones, lincosamides, streptogramins A, and phenicols, acting on the peptidyl transferase center [139].

Conclusion

In addition to their primary contribution to the metabolic process of the host, the gut microbiome is also crucial for modulating the efficacy of oral and systemic drugs routinely used in the health sector. Recent advances in multi-omics platforms, germ-free animal models, and in vitro mechanistic experiments are assisting in the identification of microbial functions and the exploration of the molecular mechanisms underlying the microbiome-drug metabolism axis. This knowledge can greatly help translational research of microbiota-targeted interventions, increase drug efficacy, avoid drug toxicity, and improve metabolic health.

Box 1: Questions of scientific and clinical interests

(i)Do gut microbial load, richness, diversity, dynamics, and genomic content be used to predict the efficacy of a drug and therapeutic duration of an acute or chronic disease?

(ii)How to select a correct sample and specific site of gastrointestinal tract to identify microbial taxa which has maximum impact on the drug metabolism and drug efficacy?

(iii)Is there any simple and robust method to detect and differentiate drug metabolites in the systemic circulation derived from host or microbes-mediated modifications?

(iv)How to select a suitable animal model and analysis method to screen microbiome-drug interactions and predict drug toxicity?

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Declarations

Competing Interests The authors declare no competing interests.

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