

# **Gut Reactions: The Importance of Gut Microbial Effects on Drug Metabolism and Disposition**

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An oft-overlooked yet critical aspect of patient responses to drugs is the role of the gut microbiota. The microbiota functions both directly and indirectly in drug metabolism, performing reductive and hydrolytic biotransformations as well as modulating host metabolic enzyme activity. As demonstrated in a recent pharmacometabonomic study of simvastatin response, microbe-derived metabolites may act as biomarkers for predicting therapy outcomes and thus allow enhanced personalized treatment regimes. A second study showcases the value of addressing microbe-host metabolic relationships in improving the therapeutic index of irinotecan. Taken together, these studies provide lessons for the pharmaceutical industry in methods of accounting for microbiota composition during drug development. Applied in parallel with metagenomic platforms and more traditional preclinical models, pharmacometabonomic analysis of gut microbial contributions to host metabolism promises to allow both improvement in drug efficacy and manipulation of the gut community for more favorable drug responses and reduced adverse drug reactions.

## INTRODUCTION

The human gastrointestinal (GI) tract harbors an immense and diverse community of symbiotic microorganisms. The metabolic capacity of this gut microbiome, which contains at least 100 times the number of genes in the human genome, invites its description as a virtual organ-within-an-organ that influences such essential host processes as gut maturation and mucosal immunity [1]. In recent years, the gut microbiota has received attention as a key mediator of host metabolism, with specific bacterial groups linked to the pathophysiology of obesity [2]. It is thus unsurprising that the gut community also participates in the biotransformation of drugs and other xenobiotics, leading to downstream (and frequently unpredicted) effects on disposition and toxicity that are imperative to consider during drug development and testing. While oral drugs that exhibit poor solubility and/or poor permeability appear to be obvious candidates for microbial transformations because of their prolonged presence in the GI tract, all drugs have the potential to reach the gut lumen via secretion/excretion, diffusion, or enterohepatic recirculation. In contrast to mammalian tissues, which mediate mainly oxidation or conjugation metabolic reactions, gut bacteria tend to engage in reduction or hydrolysis reactions, including decarboxylation, dehydroxylation, and deamination [3]. Drug metabolism by the gut microbiota may result in the production of compounds that are toxic or exhibit diminished pharmacological activity. For example, the cardiac glycoside digoxin can be converted by enteric *Eubacterium lentum* into reduced metabolites possessing much lower bioactivity [3,4]. Additionally, microbiota-derived drug metabolites may affect the host by altering the activation of host metabolic enzymes or by competing for drug transporters/detoxifying enzymes, escalating the possibility of adverse side effects or drug-drug interactions [4]. Recent studies have furthermore implicated the gut flora in hydrolyzing bile salts into their corresponding aglycones, thereby perturbing the enterohepatic recycling of biliary excreted drugs [5,6].

In short, the gut microbiota demonstrates enormous capacity for contributing to the host metabolome, with particular consequences for drug absorption, distribution, metabolism, and excretion. Since the composition (and hence overall metabolic activity) of the gut community varies according to host geographical location and lifestyle [7], the gut microbiome represents a source of non-genetic interindividual differences that could affect drug treatment outcomes. Just as pharmacogenomics has become a powerful tool for predicting individual patient responses to medication, allowing a certain degree of personalized therapy and reduction in adverse drug reactions due to genetic variation [8], the study of gut microbial effects on drug metabolism should provide similar benefits once integrated into the drug development process. An individual's microbial composition, manifest as the balance between toxic and detoxifying metabolic reactions for that individual, may very well translate into the difference between a safe, effective drug response and an adverse drug reaction. Moreover, many unexplained idiosyncratic drug reactions may actually be linked to the host's gut microbiota [6,9]. Current technologies for studying gut bacterial involvement in drug disposition include pharmacometabonomics, the prediction of drug responses using metabolic phenotypes [10,11], and metagenomics, the *in situ* study of microbial communities [12-14]. As of yet, no proposal exists for a systematic, integrated approach combining the two techniques for the purposes of minimizing toxicity and maximizing efficacy during drug development and administration. Building on two recent cases that highlight the importance of gut microbiota in drug responses—one demonstrating the utility of pharmacometabonomics in human subjects [15] and one using mechanistic knowledge of bacteria metabolism to improve drug response [16]—this paper presents one possible framework for how drug developers can address the influence of gut microbial metabolism.

## **PHARMACOKINETICS AND PHARMACODYNAMICS: SIMVASTATIN**

In the first case, Kaddurah-Daouk *et al.* use pharmacometabonomics to investigate possible effects of the gut microbiota on patient responses to the hypolipidemic drug simvastatin [15]. Simvastatin belongs to the statin family, a drug class that is used to treat hypercholesterolemia by competitively inhibiting the enzyme HMG-CoA reductase, which catalyzes the rate-limiting step of cholesterol synthesis. The main cause of hypercholesterolemia is the presence of elevated cholesterol-transporting low-density lipoprotein (LDL) levels in the bloodstream: hypercholesterolemia is, in turn, a high risk factor for atherosclerosis and cardiovascular disease. By lowering cholesterol synthesis within cells, simvastatin activates sterol regulatory element binding protein-mediated transcription of the LDL receptor gene. Increased expression of LDL receptors results in increased LDL uptake, reducing plasma concentrations of LDLs and their cholesterol cargo (LDL-C) and thereby mitigating hypercholesterolemia and associated risks [17].

Simvastatin is taken orally as an inactive lactone that undergoes hydrolysis to its active  $\beta$ -hydroxyacid form, simvastatin acid. Maximal plasma concentration occurs around four hours after drug intake, and approximately 95% of simvastatin and simvastatin acid is bound to plasma proteins. Due to extensive first-pass metabolism in the liver (as a substrate of CYP3A4), simvastatin's bioavailability is less than 5%. Further hydrolysis rapidly converts the drug to its 6'-hydroxy, 6'-hydroxymethyl, and 6'-exomethylene derivatives, all of which retain activity. Simvastatin is eliminated from the body in both the feces (60%) and urine (13%) [18].

Statins as a class exhibit on-target toxicity that presents clinically as rhabdomyolysis, i.e. muscle cell damage leading to myoglobin release and ultimately, renal failure. The postulated mechanism of toxicity is the same as the therapeutic mechanism: whereas inhibition of cholesterol synthesis in hepatocytes alleviates hypercholesterolemia (the liver is a major site of cholesterol synthesis), inhibition of cholesterol synthesis in myocytes is detrimental because it decreases concentrations of mevalonate, the immediate product of HMG-CoA reductase activity. Since mevalonate is a precursor for isoprenoid synthesis, reduced mevalonate levels lower protein prenylation in the muscle cells, attenuating protein-membrane interactions and hence diminishing mitochondrial functions that are critical for muscle cell maintenance [19]. Interestingly, simvastatin was previously a subject of a pharmacogenetics study that implicated one single-nucleotide polymorphism (SNP) in the *SLCO1B1* gene encoding drug transporter OATP1B1 as being responsible for 60% of simvastatin-induced myopathy cases. OATP1B1 mediates hepatic uptake of simvastatin, so genetic polymorphisms that reduce OATP1B1 activity result in increased plasma area under the curve, which allows greater opportunity for myocyte uptake of the drug and concomitant toxicity [20]. Bringing together *SLCO1B1* polymorphisms, gut bacteria-produced secondary bile acid levels, and response to simvastatin treatment, Kaddurah-Daouk *et al.* use a metabolomics platform to uncover a link between the three factors that will be further explored in the Discussion section.

## **PHARMACOKINETICS AND PHARMACODYNAMICS: IRINOTECAN**

In the second case, Wallace *et al.* show that inhibiting a bacterial metabolic enzyme improves therapy with irinotecan, a semisynthetic camptothecin analog used mainly against colorectal carcinomas [16]. Irinotecan binds to the DNA replication enzyme topoisomerase I when it is in complex with DNA, allowing the enzyme to make single-stranded nicks in the DNA to relieve torsional strain but preventing ligation of the breaks. This interruption of the catalytic cycle leads to double-stranded DNA damage and subsequent apoptosis. One of the hallmarks of cancer is sustained proliferative signaling with consequential uncontrolled tumor growth [21], so enzymes

involved in DNA synthesis are often highly expressed in cancer cells. In particular, colon cancer cells tend to overexpress topoisomerase I: thus, irinotecan has some selectivity for its target [22].

Irinotecan is infused intravenously as a water-soluble prodrug that undergoes tissue and serum carboxylesterase-mediated cleavage to release the active metabolite SN-38. Maximum plasma SN-38 concentration is attained approximately one hour after administration, and between 30 and 68% of irinotecan binds to plasma proteins. In contrast, ~95% of the markedly more lipophilic SN-38 is plasma protein bound. The liver UDP-glucuronosyl transferase 1A1 converts SN-38 to an inactive glucuronide, which is excreted through the bile. Hence, patients with genetic polymorphisms leading to decreased UGT1A1 activity often experience toxicity as a result of increased drug bioavailability; patients known to be homozygous for such mutant alleles are recommended to take reduced doses. Urinary excretion also contributes to elimination of the drug (~15% of irinotecan; <1% of SN-38, 3% of SN-38 glucuronide) [23].

A major dose-limiting adverse reaction to irinotecan is severe chronic diarrhea, which occurs in up to 40% of treated patients and can lead to life-threatening dehydration. Mechanistically, toxicity arises because  $\beta$ -glucuronidases expressed by the gut microbiota hydrolyze biliary excreted SN-38 glucuronide, reactivating SN-38 in the intestines. As a result of extended exposure to SN-38, healthy intestinal tissue becomes damaged in the same manner as tumor cells, and severe diarrhea occurs [24]. Broad-spectrum antibiotics have been administered to ameliorate this side effect, but antibiotic treatment has its own drawbacks, which include increased susceptibility to opportunistic infections and dysbiosis (microbial imbalance)-induced adverse gastrointestinal effects ranging from mild diarrhea to pseudomembranous colitis [25,26]. Wallace *et al.* take an elegant, more specific approach to preventing bacterial  $\beta$ -glucuronidase-related toxicity that invites contemplation in the latter part of the Discussion section.

## DISCUSSION

Thanks to rapid development of various “omics” technologies over the past several years, the necessary tools for investigating and manipulating gut microbial impact on drug disposition are readily available for use during drug development and therapy. Central to this analysis is the field of pharmacometabonomics, which allows the prediction of human drug metabolism from assessment of bacterial (and other) metabolites. Advantages of pharmacometabonomics include noninvasive sampling (metabolites can be detected in blood plasma and urine), integrated readout of environmental and genetic effects (in contrast to pharmacogenomics), and the ability to discover novel biomarkers using untargeted approaches [11].

As an example, Kaddurah-Daouk *et al.* used a targeted gas chromatography/tandem mass spectrometry platform to identify a pre-treatment metabolic profile that correlates with plasma LDL-C lowering responses to simvastatin. Informed by knowledge of the drug’s mechanism of action, the authors focused on detecting plasma metabolites related to cholesterol synthesis, dietary sterol absorption, and bile acid formation. Correlation matrix testing identified the microbe-produced secondary bile acids lithocholic acid (LCA), glycolithocholic acid (GLCA), and tauroolithocholic acid (TLCA) as showing negative correlation with reduction of LDL-C in so-called “good” responders as well as several other primary and secondary bile acids that correlate with LDL-C reduction in a wide range of responders (good, normal, poor). Of additional note is the observation that pre-treatment levels of coprostanol, a product of cholesterol hydroxylation by a subset of gut bacteria, correlate negatively with LDL-C reduction. The authors were aware of the *SLCO1B1* SNP affecting simvastatin toxicity, so they genotyped the subjects and further identified an association between the myopathy-related minor allele and

higher levels of LCA and TLCA. Their findings suggest a shared contribution of individual microbiome and genotype to simvastatin treatment outcomes [15].

Though perhaps not as rigorous as the pioneering paper of the pharmacometabonomic field [11], Kaddurah-Daouk *et al.*'s study considers the additional aspect of pharmacogenomics and offers several lessons for the pharmaceutical industry. First, the experimental scheme of acquiring pre-treatment metabonomic data and finding correlations between the data and post-treatment responses is a useful template that should be incorporated into the drug development process. Almost any metabonomic platform can be chosen for the pre-treatment analysis, with high-resolution nuclear magnetic resonance spectroscopy and high-performance liquid chromatography/tandem mass spectrometry being two other powerful possibilities. This flexibility allows existing equipment to be used for analysis and expands the sample types that may be examined: both targeted and untargeted methods may be employed. A variety of principal components analysis algorithms may then be executed to locate correlations between pre-dose metabolite profiles and treatment outcomes. Second, as genetics also frequently plays a large role in influencing drug response, simultaneous consideration of pharmacogenomic and pharmacometabonomic data would be a rational approach; an added benefit is that the same biofluid samples could be used for both analyses, so inconvenience to the patients would be minimized. The pharmacogenomic data would help dissect the pharmacometabonomic data. For example, Kaddurah-Daouk *et al.*'s observation that higher LCA and TLCA levels correlate with a transport-deficient *SLCO1B1* allele and with reduced LDL-C lowering after simvastatin treatment suggests that those bile acids may also be substrates of OATP1B1. Thus, LCA and TLCA could be competing with simvastatin for transporter binding, altering the drug's pharmacokinetics/pharmacodynamics and toxicity. GLCA levels may indicate another mechanism through which the gut microbiota influences drug disposition, bringing up the third lesson—that there is a pressing need to elucidate causative links between the two factors. While Kaddurah-Daouk *et al.* discuss possible ways in which bacteria-derived metabolites may modulate drug treatment outcome (i.e. through interactions with shared metabolic enzymes and/or transporter proteins), their study does not investigate experimentally the actual pathway(s) involved. The study's findings are therefore limited to highlighting drug response biomarkers and cannot be applied to improving drug therapy through active interventions.

Indeed, the ability to manipulate the gut microbiota for more favorable drug responses should not be underestimated, and Wallace *et al.* provide indisputable evidence supporting the utility of this approach. Knowing that bacterial  $\beta$ -glucuronidases are responsible for dose-limiting toxicity during irinotecan therapy, the authors isolated four potent and selective inhibitors of *E. coli*  $\beta$ -glucuronidase that are nevertheless nontoxic to both bacterial and mammalian cells. Co-administration of the  $\beta$ -glucuronidase inhibitors with irinotecan in mice resulted in ameliorated diarrhea compared to mice given irinotecan alone, and damage to the gut mucosa of the  $\beta$ -glucuronidase inhibitor + irinotecan-treated mice was much less severe than that of the irinotecan-only treated mice, suggesting that inhibition of the drug toxicity-perpetrating pathway is sufficient to reduce adverse drug responses [16]. The  $\beta$ -glucuronidase inhibitors are a marked improvement over antibiotics because they decrease irinotecan side effects but do not perturb the microbial balance of the gut community; thus, they present an important proof-of-principle for the design of mechanistic knowledge-based enhancements to current drug therapies.

It is not far-fetched to imagine that other gut microbiota-associated drug toxicities can be prevented by such targeted treatments, or that microbiome composition can be skewed toward a state that remains healthy, yet provides patients with increased benefit from drug therapy. Since

some gut microbes are actually capable of secreting antioxidant and other palliative metabolites [5], “second-hand treatment” in which the gut microbiota is drugged to encourage production of host-beneficial metabolites is also within the realm of possibility. Knowledge of gut bacteria metabolic contributions at the genus- and species- levels would thus be invaluable for drug developers. Unfortunately, despite the fact that the gut microbiota has been implicated en masse in a variety of drug biotransformations, the involvement of specific members of the gut community remains unclear in most cases: until recently, connections between gut bacteria and drug disposition were largely inferred from comparing drug pharmacokinetics and detected metabolites in germ-free versus conventionally colonized animals [3,4,27]. The advent of high-throughput methods for metagenomics offers the analytical power needed to uncover novel mechanisms underlying species-specific microbe-host metabolic relationships. Metagenomics aims to characterize genetic data obtained from environmental samples (e.g. the human GI tract), which allows examination of both the microbial community as a whole and individual members of the community. Mature metagenomic technologies that can be applied to the study of microbial connections to host metabolism include pyrosequencing of the conserved bacterial 16S rRNA gene and microarray analysis to identify changes in pathway activation [12,13,28,29]. A handful of studies have integrated metagenomic and pharmacometabonomic methods to identify bacterial species/groups or specific bacterial genes that covary with host metabolic profiles [12,30], but so far, none has exploited that knowledge for the rational design of treatments (e.g. probiotics, prebiotics) to alter microbial activity and thus, host response. Further experiments similar to that of Wallace *et al.* therefore need to be performed using metagenomic data for therapeutic applications. In addition, there exists an array of complementary experimental systems for assessing host-gut microbial drug metabolic interactions, including co-incubation of drugs with cecal contents/gut bacteria and hepatocytes, and comparative investigation of drug effects on germ-free animals colonized with different human microbiota compositions [4,31]. These *in vitro* and *in vivo* approaches are well worth including in preclinical drug development studies for early characterization of potential microbial influences on treatment outcomes.

Ultimately, given the arsenal of polished tools available at relatively low cost and requiring minimal technological expertise [3,31,32], pharmaceutical companies would be wise to integrate pharmacometabonomic and metagenomic analyses in both preclinical and clinical testing stages of drug development. For example, concurrent pre- and post- dose measurements of host metabolites via HPLC/MS/MS and gut community gene expression via microarrays, along with monitoring of drug response, would allow the identification of bacterial genes associated with desirable and unfavorable therapy outcomes. Metabolites exhibiting covariation with identified genes could give further clues to or validate the genes’ function; the genes could then form the basis for specific mechanistic intervention. This sort of combined analysis would assist in decreasing the likelihood of adverse drug reactions due to gut microbial effects, as well as advance the development of personalized medicine based on microbiotic (and genetic) profiles. Moreover, the elucidation of molecular and species-level connections between gut bacteria and host metabolism would make possible the manipulation of gut microbial composition to promote human health and maximize positive drug therapy responses. Finally, since the balance within the GI microbial community can be shifted by such environmental factors as antibiotic use, diet, or illness, these factors should also be considered in any study of microbe-host relationships. The recent work of Kaddurah-Daouk *et al.* and Wallace *et al.* lays the foundation for incorporating information about gut bacterial impact on human metabolic profiles, providing a compelling argument for the utility and potential of such as-yet frequently overlooked investigation.

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