PERSPECTIVE

Tiny Gatekeepers: Microbial Control of Host Drug Transporters

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Understanding interindividual variations in drug metabolism and disposition is critical to reduce patient morbidity and mortality. The microbiome contributes to such variations in part through the direct metabolism of drugs; however, the ability of host-associated microbial communities to control other aspects of drug disposition, such as absorption, remains poorly understood. Here, we highlight recent studies implicating microbes in the control of host drug influx and efflux transporters and discuss key areas for future studies.

The absorption of orally administered drugs is driven by the net effect of the intrinsic physicochemical properties of a given drug, the physiological conditions of the gastrointestinal tract, and the activity of drug transporters.[1] Transporters, as gatekeepers and protectors of the cells stationed on the plasma membranes of enterocytes, control both the influx and efflux of drugs.[1] Two main superfamilies of transporters are solute carrier family (SLC) that primarily aid in drug uptake (but can sometimes perform drug efflux) and ATP-binding cassette (ABC) transporters that mainly aid in drug efflux.[1] Among more than 300 members in the SLC transporter superfamily, key intestinal transporters include the organic anion transporting polypeptide (OATP) family and peptide transporter 1 (PEPT1/SLC15A1).[2] OATPs transport diverse substrates that include bile acids, peptides, and xenobiotics (foreign compounds like pharmaceuticals) by coupling their influx with the efflux of endogenous intracellular substrates to maintain electrical neutrality.[1,2]

In contrast, ABC transporters often protect cells through drug efflux, delaying absorption in the gastrointestinal tract and elevating drug concentrations in the gut lumen.[2] Among the 49 ABC genes in humans, P-glycoprotein (P-gp encoded by the *ABCB1* gene), breast cancer resistance protein (BCRP encoded by the *ABCG2* gene), and multidrug resistance protein (MRP2 encoded by the *ABCC2* gene), are well-studied transporters that limit absorption of a wide range of structurally diverse compounds.[2] Cancer cells take advantage of this defense mechanism and upregulate *ABCB1* expression to lower intracellular drug concentrations, rendering anticancer drugs less effective.[2] Changes in transporter expression can result in potentially fatal drug–drug interactions, as shown for the highly toxic cardiac glycoside digoxin. Coadministration of digoxin with a P-gp inducer led to subtherapeutic serum concentrations of digoxin in patients while coadministration with a P-gp inhibitor led to digoxin toxicity.[1] Edible plants such as green tea leaves and St John's wort can also modulate P-gp activity.[1] Given the importance of these efflux transporters in drug disposition, the US Food and Drug Administration recommends testing any new drug for the capacity to modulate P-gp and BCRP.[2]

Understanding the full scope of genetic and environmental factors that modulate the expression and function of SLC and ABC transporters is essential due to the critical role these transporters play in shaping drug absorption. However, despite prior data implicating the microbiome in transporter gene expression,[3,4] there is still limited insight into the molecular and cellular mechanisms responsible, or their downstream consequences for transporter activity and treatment outcomes. RNA sequencing analyses of intestinal tissues from germ-free mice and conventionally raised (CONV-R) mice showed that the gut microbiota modulates the expression of 11 uptake transporters and 6 efflux transporters.[3] Of note, *Slco2a1* (which encodes Oatp2a1) and *Slc15A1* (Pept1) uptake transporter genes in the SLC transporter superfamily had significantly lower jejunal and colonic expression in CONV-R mice. *Slco3a1* (Oatp3a1) had significantly higher jejunal expression in CONV-R mice.[3] Twenty-four transporter genes including *Slco2b1* (Oatp2b1), *Abcb1a* (P-gp), *Abcc1* (Mrp1), and *Abcg2* (Bcrp) were not differentially expressed.[3] However, a prior study had shown that CONV-R mice have significantly decreased jejunal and ileal *Abcb1a* expression,[4] suggesting

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Received April 14, 2022; accepted May 8, 2022. doi:10.1002/cpt.2647

gut microbial communities may vary in their ability to alter host transporter gene expression.

Additional support for differences between gut microbial communities in their ability to shape host drug transporters comes from antibiotic perturbation experiments. Mice were treated with a panel of antibiotics, resulting in a significant decrease in colonic P-gp protein levels in response to vancomycin.[5] This phenotype was transmissible via the gut microbiota of vancomycin-treated donor mice to antibiotic-depleted CONV-R recipient animals. Colonic P-gp protein levels were associated with the abundance of members of the Firmicutes phylum and bacterial genes to produce short-chain fatty acids, including butyrate.[5] Butyrate was also sufficient to increase P-gp protein levels in a human colorectal cancer cell line.[5] These results extend prior work on secondary bile acids as potent inducers of the pregnane X receptor (PXR) and *Abcb1a* expression,[5] suggesting that additional bacterial metabolites like butyrate can contribute to changes in colonic protein levels. More work is needed to assess the generalizability of these findings to the small intestine and other organs, the functional consequences of butyrate and/or other gut bacterial metabolites for drug efflux, and the ability of these pathways to shape drug pharmacokinetics and pharmacodynamics.

Like mammalian cells, bacteria encode their own transporters that may contribute to the sequestration of drugs, limiting the ability of these compounds to reach general circulation. For example, *Escherichia coli* has multiple transporters that mediate the uptake of peptides and peptidomimetic drugs like the human PEPT1 transporter.[6] A recent study used caffeine, which contains a peptide bond and is similar in size to a dipeptide, to probe the drug absorption capacity of the gut microbiome.[6] Caffeine was detected in the gut microbial pellet at 2–5 hours post oral administration in rats,[6] suggesting that the microbiome sequesters caffeine prior to intestinal absorption. Similarly, a high-throughput screen of 15 drugs and 25 human gut bacterial strains identified 4 drugs, including the antidepressant duloxetine, that showed signals of bioaccumulation within bacterial cells without metabolism.[7] Bacterial preincubation with duloxetine interfered with the ability of the drug to inhibit *Caenorhabditis elegans* motility.[7] Taken together, these studies highlight the importance of considering active transport and cell surface binding to microbial cells following oral administration.

The microbiome may also shape drug absorption through its extensive interactions with food and drug additives. We recently showed that the azo bond containing dye FD&C Red No. 40 is a potent inhibitor of the OATP2B1 uptake transporter.[8] Coadministration of FD&C Red No. 40 with the OATP2B1 substrate fexofenadine (an antihistamine) led to decreased drug bioavailability in mice.[8] Interestingly, human gut microbial communities and bacterial isolates were capable of depleting FD&C Red No. 40 and other related azo dyes that inhibit OATP2B1.[8] The predicted downstream bacterial metabolites and spent media were no longer able to inhibit transporter function,[8] suggesting that gut bacterial metabolism of these common food and drug additives rescues their impact on intestinal absorption. Another recent study looked at the gut bacterial metabolism of ellagic acid and ellagitannins (found in fruits like pomegranates and strawberries) into urolithins, which are the substrates of the BCRP efflux transporter.[9] Urolithin A inhibited transport of a BCRP substrate mitoxantrone (an anticancer drug) via competitive inhibition leading to increased intracellular concentration of mitoxantrone in a dose-dependent manner.[9] Taken together, these studies illustrate that the microbiome can both ameliorate and exacerbate the impact of components of our diet on host drug transporters.

Importantly, the microbiome can also biosynthesize a plethora of small molecules that could impact host drug transporters. Bioinformatic analysis of bacterial reference genomes and microbiomes has emphasized this vast biosynthetic potential.[10] One of the biosynthetic gene clusters identified produced a molecule referred to by the authors as wexrubicin, which was highly similar in structure to the anticancer drug doxorubicin, a well-known substrate of the P-gp efflux transporter.[10] Thus, it would be interesting to test whether these microbial metabolites interfere with doxorubicin efflux and if so, whether interindividual variations in their production affect cancer treatment outcomes. More broadly,

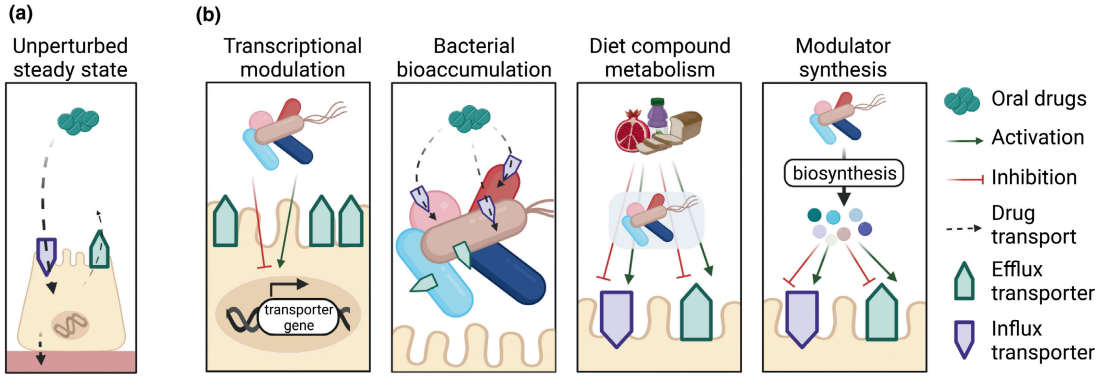

Figure 1 General mechanisms through which the microbiome impacts host drug transport. (**a**) A simplified model of drug absorption without the effect of the gut microbiome. (**b**) Known mechanisms of microbiome–transporter interactions, including microbial control of host gene expression and/or protein levels, bacterial sequestration and bioaccumulation of drugs, biotransformation of dietary components that interact with drug transporters, and biosynthesis of microbial metabolites that affect transporters. Created with BioRender.com.

concerted efforts are warranted to search for and characterize additional microbial metabolites that interact with host drug transporters.

In conclusion, these early studies support multiple general mechanisms through which the microbiome modulates drug absorption, including (i) microbial control of host gene expression and protein levels; (ii) bioaccumulation of drugs in microbial cells; (iii) microbial biotransformation of dietary substrates; and (iv) biosynthesis of microbial metabolites (Figure 1). Far more research is needed to understand the mechanistic details of each type of host-microbiome interaction coupled to the development of high-throughput screening platforms, experimentally tractable co-culture systems that permit the analysis of direct interactions between host and microbial cells, and a more focused attempt to assess these phenomena in human cohorts. A key gap in knowledge is the effect size of the microbiome relative to more established mechanisms like host genetic sequence variations; can the microbiome have comparable effects relative to complete and/or partial loss-of-function alleles? How does the magnitude of microbial metabolite-induced changes to drug transport compare with previously reported drug–drug interactions? Importantly, not all drugs are sensitive to subtle differences in concentration so it will be important to prioritize compounds with a narrow therapeutic range or the highest magnitude of microbiome dependency. Another major knowledge gap is the role of other microorganisms in host drug absorption, including fungi, parasites, and viruses, which could be readily studied in CONV-R or gnotobiotic mouse models, ideally in the context of transgenic mice that have been "humanized" to express the same transporters found in human cells. There is much to be done, but the wide-open nature of this research area promises many fundamental insights into both humans and our associated microorganisms. Continued progress in this area will be a critical step toward collaborating with our tiny gatekeepers to more precisely predict and control drug disposition.

FUNDING

This work was supported by the National Institutes of Health (R01HL122593; F30CA257378). P.J.T. is a Chan Zuckerberg Biohub Investigator.

CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

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