Gut Microbes Take It to the Next Level? First Insights Into Farnesoid X Receptor Agonists of Microbial Origin

The intestinal microbiome and its metabolites contribute to host (patho)physiology through signaling via host bile salt receptors. To assess the overall chemical effects of the microbiome at the level of an entire animal, Quinn et al. conducted untargeted metabolome analysis of 29 different organs of germ-free and specific pathogen-free (SPF) mice. Unique microbial and metabolome profiles were evident along the gastrointestinal (GI) tract. An elegant mass spectrometry informatics approach led to the identification of three novel bile salt conjugates that were present along the entire intestinal length of SPF mice only, with highest levels observed in the small intestine. Specifically, N-amidates of cholic acid (CA) and either leucine (Leu-CA), phenylalanine (Phe-CA), or tyrosine (Tyr-CA) were identified and shown to be produced by specific strains of Clostridium bolt-eae. Administration of these new bile salt conjugates to mice resulted in changes in ileal and hepatic gene expression compatible with local activation of the bile salt receptor, farnesoid X receptor (FXR). Mining of public databases resulted in spectral matches to the novel bile salt conjugates in GI tract/fecal samples of patients with Crohn’s disease and in cystic fibrosis.

Bile salts are synthesized in the liver. Before their release in bile, bile salts undergo conjugation (i.e., N-amidation) with glycine or taurine to form molecules (glycocholic acid [GCA] and taurocholic acid, respectively) that are largely negatively charged at ambient pH in their enterohepatic trajectory. Conjugation increases aqueous solubility of bile salts and limits passive uptake in the proximal small intestine before completion of their digestive function. Among naturally occurring amino acids, glycine and taurine serve as exclusive conjugation partners, likely relating to their overall resistance to cleavage by pancreatic carboxypeptidases. A stable pool of bile salts is maintained in enterohepatic circulation with dedicated membrane transporters in the ileum and liver. Only a small portion of the bile salts that enter the duodenum after a meal spills over to the caecum and colon where the bulk of microbial bile salt transformation occurs. Reactions include deconjugation, a feature shared by a wide range of bacteria, and several modifications of the steroid nucleus, which are restricted to a number of bacteria including Clostridium species. The study by Quinn et al. is the first demonstration of bile salt conjugation by nonhost enzymes. It is well established that bile salts are potent signaling molecules that interact with dedicated receptors at the plasma membrane (e.g., Takeda G-protein-coupled receptor 5 [TGR5]) or inside the cell (e.g., FXR), to control numerous metabolic processes. Microbial metabolism of host bile salts results in diversification of the circulating bile salt pool and impacts on host signaling through bile salt receptors.

The observation of bile salt conjugates of microbial origin is intriguing, if only because the gut microbiota are primarily implicated in catabolic breakdown of dietary components rather than in anabolic conversions. The investigators convincingly demonstrate that the novel conjugates are not host-derived, but formed by gut bacteria. Their abundance (Phe-CA > Tyr-CA > Leu-CA) was highest in the jejunum and ileum. Possible bacterial producers include specific strains of C. bolt-eae, which were shown to conjugate free CA with the respective amino acid precursors. Note that >99% of the bile salts in bile are conjugated with glycine or taurine. Although luminal amino acids are abundant in the digestive phase, the source of free CA as substrate for microbial conjugation is unresolved. Free CA could originate from the small fraction of non-conjugated bile salts present in bile, limited deconjugation of GCA by pancreatic carboxypeptidases, or deconjugation by abundantly present microbial bile salt hydrolases. It remains to be determined whether additional amino acids, or different bile salt species, are (co)substrates for bacterial formation.
of bile salt conjugates. The investigators ruled out occurrence of atypical conjugates of muricholic acids, abundant bile salt species in mice that cannot be discriminated from CA by mass spectrometry only.

Microbial deconjugation and further conversion of bile salts is proposed to reduce bactericidal action of bile salts by lowering solubility in fecal water. Why does C. bolteae, a member of a genus that is known to tolerate bile, form these new bile salt conjugates? Addition of the new conjugates to actively growing human fecal cultures had no effect on microbial community structure, suggesting that they do not act as a niche factor to suppress growth of bacteria sensitive to conjugated bile salts. Another possibility is that bacterial conjugation protects certain Clostridia species from growth-inhibitory effects of free bile salts. Targeted analysis revealed that levels of the new conjugates were >10-fold lower in colon or feces than in the small intestine. It is likely that bacterial deconjugation of the novel conjugates occurs in view of earlier observations. Furthermore, bacterial conjugates, similar to their host counterparts, could be actively reclaimed in the ileum for return to the liver. All three bacterial conjugates apparently meet substrate preferences (i.e., single negatively charged bile salts) for uptake from the intestinal lumen (by apical sodium-dependent bile acid transporter) and portal circulation (by sodium taurocholate cotransporting polypeptide), with enterocyte release into the portal blood being conceivable given the substrate promiscuity of organic solute transporter α/β. Yet, none of the novel conjugates were detected by a sensitive assay in portal blood, liver tissue, or gallbladder bile of experimental animals. This argues against these compounds having an enterohepatic circulation. Possible deconjugation to free CA may occur by intestinal or pancreatic (carboxy)peptidases. Hence, it is currently unclear whether bacterial bile salt conjugates enter ileal cells and beyond in sufficient levels for local activation of intracellular bile salt receptors or have a restricted luminal action.

The investigators examined the potential of the novel conjugates to activate FXR in a cell reporter assay and in mice. Although the choice for a kidney-based cell system with uncertain expression of transporters engaged in uptake of bile salt ligands is less obvious, dose-dependent activation of FXR was observed for Phe-CA and Tyr-CA, with the latter having the highest potency (half-maximal effective concentration \([EC_{50}] = 0.14 \, \mu\text{mol/L}\) that outranked the most potent endogenous bile salt (viz., chenodeoxycholic acid; \(EC_{50} = 9.7 \, \mu\text{mol/L}\)). Gavage of the microbial bile salt conjugates resulted in induction of FXR target genes in ileum and liver, along with anticipated repression of bile salt synthetic genes. Comparable effects were observed when administering an identical dose of CA. The lack of measurements in portal/systemic blood precludes a conclusion on absorption of the bacterial conjugates in intact form. Given the clinical interest in agonistic FXR activation for treatment of metabolic (nonalcoholic steatohepatitis) or cholestatic (primary biliary cholangitis, primary sclerosing cholangitis) liver diseases, further investigation into the enterohepatic dynamics of these novel bile salt conjugates is warranted. Likewise, their interaction with plasma membrane receptors for generic (un)conjugated bile salts (i.e., TGR5) or particular conjugated bile salt species (e.g., sphingosine-1-phosphate receptor 2, muscarinic receptors) should be explored.

Analysis of public metabolome data sets revealed spectral matches to the novel conjugates in fecal samples of healthy persons and patient populations. Their presence was more frequent in pediatric patients with cystic fibrosis and pancreatic insufficiency resulting in fat malabsorption. Likewise, increased abundance of each of the three bacterial conjugates was noted in Crohn’s disease, but not in ulcerative colitis, and this was associated with a dysbiotic state. It is conceivable that dysbiosis (e.g., through small intestinal bacterial overgrowth) could result in increased bacterial de- and reconjugation of bile salts. Feeding normal or atherosclerosis-prone mice a high-fat diet caused elevation of fecal levels of bacterial bile salt conjugates, underlining an apparent dysbiotic link, and providing a rationale for analyses in patients with obesity-related disorders. It will be worthwhile to explore whether the microbial bile salt conjugates contribute to dysbiosis or are the consequence thereof and may serve as a biomarker.

In conclusion, the elaborate study of Quinn et al. brings a new twist to the established modulation of host processes by microbial bile salt metabolites. The current discovery of CA conjugates of microbial origin is only the beginning, raising more questions than are being answered. It will be key to know whether the novel conjugates are actually absorbed in humans, to levels sufficient for eliciting bile salt receptor activation.
Long-Term Perfusion of the Liver Outside the Body: Warming Up for Ex Vivo Therapies?

The term “game-changing technology” is every so often used as a hyperbole to underline the importance of research to the outside world. The experimental work of the ETH Zurich and University Hospital Zurich, recently published in Nature Biotechnology by Eshmuminov et al.,(1) is, however, no less than a real game changer in the field of liver transplantation. The authors achieved an increase in stable liver function ex vivo from a few hours to 7 days using a normothermic machine perfusion (NMP) device. This leap forward in perfusion time, while maintaining physiological balance, is incredibly important as it opens a window of opportunity to explore ex vivo organ repair therapies.

Driven by organ shortage and waiting list mortality, transplant physicians are pushed to use donor organs of marginal quality. NMP provides opportunities to test and even improve the quality of these grafts. However, with current commercially available NMP devices, safe perfusion is only warranted for up to 24 hours. In most clinically used perfusion protocols, the portal vein is perfused with highly oxygenated blood, resulting in a high demand for vasodilator medication. Furthermore, the perfusion systems lack sufficient physiological support, which is detrimental for long-term perfusion of donor livers. Obviously, in vivo, the liver is not an autonomous organ. Liver homeostasis depends on hormonal systems, such as pancreatic glucose regulation and kidney filtration to remove waste products, to regulate electrolyte levels, extracellular volume, and pH balance. Furthermore, motion created by the contraction of the diaphragm aids in perfusing the liver and provides biomechanical support for the functioning of the liver.

To achieve sustained ex vivo perfusion, in vivo physiological conditions were mimicked. Glucose levels were monitored in real time, and glucose homeostasis was automatically regulated by administering glucose, insulin, or glucagon. Furthermore, an integrated dialysis system continuously removed waste products and maintained electrolyte levels. Diaphragm movement

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