Human Microbial Metabolite Mimicry as a Strategy to Expand The Chemical Space of Potential Drugs

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Biomimicry of drug-like microbial metabolites can potentially expand our drug pipeline. This strategy could result in potent yet safe drugs, if the metabolites and their targets are carefully chosen and contextually applied to human diseases.

Abstract

The concept of small molecule mimicry even of weak microbial metabolites present in rodents and humans, as a means to expand drug repertoires, is new. Hitherto, there are very few proof-of-concept papers demonstrating utility of this concept. More recently, papers demonstrating mimicry of intestinal microbial metabolites could potentially expand the drug repertoire for diseases such as Inflammatory Bowel Disease. We opine that as more functional metabolite-receptor pairings are discovered, small molecule metabolite mimicry could be a significant effort in drug discovery.

Biomimicry is an invaluable tool for discovery of new solutions to problems afflicting humankind. Examples include the design of Velcro, which mimics burdock burrs. Shark denticles are used to design surfaces (riblets) that reduce air drag. Beehive patterning of circuits are used to balance loads during peak hours, and design of energy-conserving pools that imitate soap bubbles [1]. Another example relates to biology and predation. Several species, for instance, caterpillars use eyespots to give predators the impression of a sizeable toxic animal (e.g., snake's head) [3].

Biomimicry has been explored in therapeutics [4,5], but there is a real paucity of emerging concepts and drugs [6]. In therapeutics, biomimicry would theoretically yield potent drug-like molecules with minimal liabilities (i.e., side-effects). This assumption is made based on the similarity of the small molecule to the original parent metabolite that it mimicked, no significant metabolic liabilities, as well as overall safety in animal studies. These small molecules could potentially improve the chemical space utilized in traditional drug discovery efforts (Figure 1) [7]. We base our concepts on the debated rationale that Nature minimizes the cost of development of molecules that have the most effect on their targeted goal with minimal effects on its environment (i.e., untargeted liabilities). As an example, several invading plant pathogens (e.g., Xanthomonas), when present within their host, induce the expression of a specific protein, RaxX. RaxX is a mimic of a plant peptide hormone, PSY (plant peptide containing sulfated tyrosine). The rice immune receptor XA21 detects sulfated RaxX preferentially over endogenous peptide PSY. The binding of RaxX to XA21, in turn, results in accessing control over host signaling and immunity that protects both the host and the pathogen [8]. Sulfated RaxX-XA21

interactions are specific, and there are no known other receptors that are engaged by RaxX [9]. In this example, Nature conserves biochemistry and targets specific host proteins without collateral damage, so it ensures its pathogen-host survival. One possible conjecture is that nature-made metabolites (and proteins) that are designed to reach and influence their specific target would have limited toxicity to the host (e.g., unless they are purposefully made to be toxins, antibiotics etc.). However, caution must be alarmed as this could also simply be a co-evolution where the host has evolved to avoid toxicity from the metabolite (or protein).

Bioinspired chemical libraries are clearly changing traditional drug discovery approaches. For example, Thireou et al. used a two-filter chemical screening approach, in which in the first filter, they screened for shape and chemical structure similarity of plant-derived insect repellents. In the second filter, they screened the ligand binding mode to the Anopheles gambiae odorant binding protein (AgamOBP1) as compared to canonical ligands. The authors were able to show hits with N,N-Diethyl-meta-toluamide (DEET)-like activity but with lower volatility and increased protection time from a mosquito bite [10]. In another example, the epothilones (EpoA-EpoD) are sixteen (16)-membered macrocyclic lactones identified in 1996 [11]. Epothilones A (EpoA) and B (EpoB) are products of a soil myxobacterium, *Sorangium cellulosum*, strain So ce90 and would be considered nature-inspired compounds [11,12]. Epothilones bind to specific regions on tubulin and promote polymerization [13,14], which has been associated with its anti-cancer activity [12]. Ixabepilone (BMS247550) is an esterase-cleavage resistant analog of EpoB lactam (biomimic of EpoB), and its success in human clinical trials led to its approval by the FDA

for the treatment of metastatic breast cancer [15,16]. Similarly, newer antibiotics (e.g., thiopeptide antibiotics) have been sourced from marine and soil bacteria [17]. In lieu of a limited success for high-throughput target-based screening of chemical libraries for antibiotics, more recently, actinomycetes have been mined for antibiotics discovery via high throughput fermentation [18]. In similar fashion, several active drugs are microbial metabolites (e.g., erythromycin) and others are derivatives of macrolactone metabolites (e.g., FK520) [19]. Bacteria are now considered appropriate and important vehicles for targeted engineering to optimize production of highly bioactive compounds [7]. It is clear that natural product drug discovery has had a strong and sustained influence in drug discovery.

In our “features” article, unlike the well-established field of natural products from soil and marine organisms, we capitalize on the concept of weakly potent mammalian microbial metabolites, and our ability to generate potent yet safe small molecule mimics of these metabolites to expand the chemical space for potential drugs of the future[20]. The gut microbiome has evolved in parallel with its host for many generations and is thus very well adapted to it. The host is taking advantage of the microbiome for many key physiological functions but keeps it under control. On the other hand, the gut microbiome thrives within the host gut and modulates its physiology to its benefit. The host-microbiome interactions go in both ways, thus establishing a symbiosis state.

The microbiome-derived molecules are among the most important actors in this process and they have been demonstrated to target many host receptors and particularly G-protein coupled receptors (GPCRs) with local effects in the gut as well as systemic effects

[21]. It is thus expected and now well established that microbiota derived-metabolites influence disease (e.g., short-chain fatty acids and intestinal inflammation) [22] (Figure 2). Some authors have proposed that microbial metabolites could possess intrinsic therapeutic properties [4,23]. Microbial metabolites can also act as immune-therapeutics (activating or inhibiting immune cells that act to favorably change the course of a disease) [24]. Our evolving knowledge of how microbial metabolites influence disease now sets forward a path towards microbiome-based metabolite treatment of disease [25]. The lack of inherent drug-like features (e.g., logP, logD, molecular weight constraints) or pharmacology for systemic or prolonged local delivery limits the clinical utility of metabolites serving as drugs. For example, indole 3-propionic acid (IPA) has anti-diabetic [26], anti-tuberculosis properties [27], and may be beneficial in treating a host of inflammatory [28] and neurological disorders [29]. IPA has good drug-like and lead-like properties; however, it is relatively insoluble in aqueous formulations (0.73 g/L) [30], so for clinical delivery, water-soluble formulations have been developed [31]. Other metabolites like butyrate, which also ameliorate inflammatory disorders [32], have rapid systemic clearance, and either clinically cumbersome dosing schedules are required or a need to design stable butyrate analogs [33].

As an alternative approach, one can design smart analogs of known and well-defined interactions between microbial metabolites and host receptor proteins (e.g., short chain fatty acids or SCFAs, with histone deacetylase or HDACs or GPCRs, indole with arylhydrocarbon receptor or AhR, or IPA and pregnane X receptor or PXR) (Figure 3)[34]. Indeed, our group demonstrated that indole/indole 3-propionic acid formed by intestinal

microbes could activate PXR, which then results in the down-regulation of the TLR4-NFkB inflammation pathway in mice [28]. More recently, we have shown that small molecule mimics of indole/indole 3-propionic acid to our receptor (PXR), can deliver more potent activation of the receptor. Since the small molecule hits (FKK5/FKK6) mimic endogenous indole metabolites, it is non-toxic when compared to other known PXR xenobiotics [35](*Trends in Mol Med*, In press) (Figure 3). We are currently optimizing these small molecules as therapeutic hits.

In this context, one could develop a variety of metabolite analogs with increased potency and with a high safety margin for several diseases. Tilivalline (an indole analog) is a pathogenicity factor produced by *Klebsiella oxytoca* and known to cause antibiotic associated hemorrhagic colitis (AAHC). Indoles (simplified tilivaline analogs) are known to suppress production of tillivaline and such may be developed to counteract AAHC [36]. Urothelin A (UA) has anti-cachexia/sarcopenia [37] and anti-atherosclerosis potential [38]. While the targets of Urothelin A are several, but the potency and stability on select receptors (e.g., AhR/Nrf2) is improved with analogs like UAS03 (Figure 3) [39]. SCFA) target HDACs and GPCRs and have beneficial effects on health [40]. For example, butyrate targets receptors, FFA2 (GPR 43) and HCA2 (GPR109A). While, a very close analog, β-hydroxybutyrate targets only HCA2 (GPR109A). If this is extended to an octanoate, then specificity is achieved as it targets HCA1 (GPR81)[41]. This specifically highlights how receptor-specific analogs of the microbial metabolites maybe developed. . Thus, putting all these observations together, in our opinion, microbial metabolite-receptor or functional pairings, coupled with computational analog screening with facile chemical

synthesis methods, could open up a new chemical space for drug discovery. As investigators uncover more pathways pertinent to metabolites, examples similar to what we have shown, may hold a new key for drug discovery [35].

Disclosure

We (SM, ZD) have filed a patent application US 2019/0367475 A1 on PXR agonists and uses thereof for gut barrier dysfunction and treatment prevention.

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Figure Legends

Figure 1. Strategies to expand Chemical Space. These examples come from natural products, whose structural analogs have yielded an expanded chemical space yielding drugs for many pathophysiologic processes [42,43].

Figure 2. Schematic of Microbial Metabolites that Influence Human Health. Metabolites featured in blue ameliorate the condition noted; those in red augment or participate in the pathogenesis. Depicted organ from head to toe are the coronal section of the brain, the heart, and the intestines. The metabolites referred to here, arise from intestinal microbial metabolism. BA, bile acids; SCFA, short chain fatty acids; TMAO, Trimethyamine-N-oxide; 4EPS, 4-ethylphenylsulfate; DAT, desaminotyrosine. The figure was inspired and adapted from Descamps HC et al [23]. This figure is not a comprehensive listing or association, but an illustration of the diversity of effects by metabolites on multiple organ systems.

Figure 3. Chemical Structures of Metabolites and their Mimics. (A) Indole and indole 3-propionic acid (IPA) are illustrated and individually have very weak affinity for PXR binding and activation. A lead mimic (FKK6) of the combined pharmacophore of indole and IPA binding the PXR ligand binding domain is shown to the right of the arrow with significantly improved affinity for PXR binding. FKK6 has PXR-dependent anti-inflammatory properties in mice [35]. (B) Indole is an arylhydrocarbon receptor (AhR) agonist with EC_{50} ~ 3μM in luciferase reporter assays [44]. PY109 (EC_{50} ~1.2 nM) is a more potent and drug-like indole mimic and has demonstrated anti-inflammatory properties in mice [45]. (C) Urothelin A (UroA) is a micromolar range activator of the AhR- nuclear factor erythroid 2–related factor 2 (Nrf2) pathway. It us an unstable compound in solution. UAS03, is a stable analog of UroA and has a slightly increased potency of effect in the activation of the AhR-Nrf2 pathway. UAS03 has anti-inflammatory properties in mice [39].