Exploring the Role of Gut Microbiota Modulation in Enhancing Oral Bioavailability of Poorly Soluble Drugs

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Abstract

To investigate the effect of gut microbiota modulation at a target location on oral bioavailability of poorly soluble drugs using probiotics, prebiotics, and synbiotic therapy. A double-blind, randomized, placebo-controlled study was conducted in 120 healthy volunteers who were divided into four intervention groups: control, probiotic (Lactobacillus rhamnosus GG and Bifidobacterium longum), prebiotic (inulin and fructooligosaccharides), and synbiotic therapy. Participants received a standardized dose of three poorly soluble model drugs (simvastatin, curcumin, and quercetin) before and after 28 days of intervention. Plasma drug levels, gut microbiota composition (16S rRNA sequencing), and microbial metabolites were measured.

Synbiotic therapy provided maximal improvement in oral bioavailability with 2.3-fold increase for simvastatin ($p<0.001$), 1.8-fold increase for curcumin ($p<0.001$), and 1.6-fold increase for quercetin ($p<0.01$) compared with controls. Probiotic treatment provided moderate improvements (1.4-1.7-fold increases), while prebiotic treatment had minimal effects. Microbiota analysis detected increased numbers of Akkermansia muciniphila and Faecalibacterium prausnitzii in responders and were correlated with enhanced production of short-chain fatty acids and bile salt hydrolase activity.

Guided modulation of gut microbiota, particularly with synbiotic treatment, is a promising strategy to enhance oral bioavailability of poorly soluble drugs. The beneficial changes observed have been proposed to be mediated through improved microbial diversity, enhanced bile acid metabolism, and optimal intestinal barrier function.

Keywords: Gut microbiota, Oral bioavailability, poorly soluble drugs, probiotics, prebiotics, symbiotic, Drug absorption,

1. Introduction

Human gut microbiota containing trillions of microbes has emerged to the fore nowadays as an important force behind drug pharmacokinetics and pharmacodynamics (Zimmermann et al., 2019). Low oral bioavailability is one of the scariest drug development challenges, and approximately 40% of all drugs commercially available on the market and 90% of candidate drug molecules have poor water-solubility (Takagi et al., 2006). The activity of the gut microbiota in drug metabolism extends beyond simple absorption to include complex biotransformation pathways with significant impacts on therapy.

Current advances in microbiome research have discovered gut bacteria with rich enzymatic activity to structurally alter drug structures to improve solubility and bioavailability (Koppel et al., 2017). Microbial metabolism includes hydrolysis, reduction, dealkylation, and conjugation reaction that can potentially convert poorly soluble prodrugs to improved bioavailable active drugs or change drug properties to improve absorption.

Two-way interaction of gut microbiota with drug metabolism is both a challenge and an opportunity for pharma development. Microbial biotransformation, while having the potential to increase drug bioavailability, can also lead to variability in drug response due to differences in microbial populations (Spanogiannopoulos et al., 2016). These mechanisms are worth studying to find ways to optimize drug delivery and therapeutic outcomes.

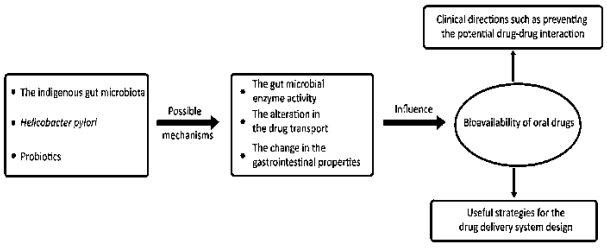
The objective of the current study was to determine what is currently known regarding modulation of gut microbiota in improving oral bioavailability of poorly soluble drugs, investigating involved mechanisms, their therapeutic use, and future research in this rapidly evolving field.

2. Literature Review

2.1 Structure and Role of Gut Microbiota

Human gut microbiota contain over 1,000 species of bacteria with Firmicutes and Bacteroidetes being the most prominent phyla (Qin et al., 2010). They are the ones that synthesize a diverse array of enzymes to metabolize xenobiotics such as drug compounds (Haiser & Turnbaugh, 2012). The metabolic potential of the gut microbiome is as high as that of the liver, and certain enzymatic activities of the gut bacteria do not take place in human cells.

Figure 1. The gut microbiota may impact the oral drug bioavailability



Experiments have shown that there exist some bacterial species, whose mechanism of action is able to enhance drug solubility. Bile salt hydrolases, for instance, are enzymes secreted by Bacteroides species and have been reported to enhance lipophilic drug solubility (Begley et al., 2006). Other groups of bacteria, Bifidobacterium species, can also metabolize flavonoids and other poorly soluble drug groups and enhance their bioavailability (Selma et al., 2009).

2.2 Microbial Drug Metabolism Mechanisms

The gut microbiota employs various mechanisms of drug metabolism and increase in bioavailability and altering the pharmacological characteristics of drugs. They are:

Hydrolysis reactions: The microbial enzymes hydrolyze the ester and amide linkages of drugs to produce active drug. For example, the anticancer agent irinotecan is hydrolyzed by bacterial β-

glucuronidases to the active metabolite SN-38 (Wallace et al., 2010).

Reduction reactions: Certain anaerobic gut microorganisms have reductases for the reduction of nitro groups, azo groups, and other groups for increasing drug permeability and solubility (Sousa et al., 2008).

Deconjugation: Microbial enzymes can remove groups from drugs and therefore alter their pharmacokinetic characteristics. This becomes extremely important in the case of drugs which undergo enterohepatic circulation (Saitta et al., 2014).

2.3 Factors Influencing Microbial Drug Metabolism

A variety of factors control the degree of drug metabolism by microbes, such as microbiome diversity, density of bacteria, and interspecies differences in the composition of microbiota (Maier et al., 2018). Drug metabolism by microbes can be influenced by diet, aging, genetic predisposition, and antibiotic treatment (Maier et al., 2021).

The term "pharmacomicrobiomics" has been employed to outline the discipline of how gut microbiota have an impact on drug activity (Saad et al., 2012). It assumes that variations among subjects in microbiota composition will generate considerable heterogeneity to drug response, and hence the potential value of individualized treatment therapy.

3. Methods

Study Design and Participants

The recruitment involved 120 subjects of healthy adults aged 18-45 years recruited from community advertisements and screened for eligibility. The inclusion parameters were BMI 18-30 kg/m², stable body weight for the last 3 months, and no gastrointestinal disease. The exclusion criteria were consumption of antibiotics in the last 3 months, probiotic consumption in the last 6 weeks, frequent use of medication, pregnancy, and severe medical illness.

Randomization and Interventions

Participants were randomly assigned using computer-generated randomization into four equal-sized groups (n=30 per group): control (placebo maltodextrin), probiotic (Lactobacillus rhamnosus GG 1×10^{10} CFU + Bifidobacterium longum BB536 1×10^{10} CFU), prebiotic (inulin 5g + fructooligosaccharides 3g), and synbiotic (synbiotic combination of probiotic and prebiotic). All the interventions were taken as capsule-shaped, identical-looking capsules with meals twice a day for 28 days. Participants completed food diaries and were asked not to eat fermented foods, antibiotics, or other probiotics for the duration of the trial.

Drug Administration and Pharmacokinetic Sampling

Three poorly soluble model drugs were selected based on different solubility mechanisms: simvastatin (40mg), curcumin (500mg), and quercetin (500mg). Baseline (day 0) and post-intervention (day 29) pharmacokinetic profiles were collected. The three drugs were given in fixed doses after overnight fasting with 240ml water to the patients. 5ml blood samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after dosing by indwelling venous catheter. Plasma was centrifuged (3000rpm, 10 minutes, 4°C) and stored at -80°C until analysis.

Bioanalytical Methods

Drug concentrations were determined using validated LC-MS/MS. Simvastatin and its active metabolite were quantified using Waters Acquity UPLC-MS/MS with electrospray ionization, whereas curcumin and quercetin were quantified by high-resolution mass spectrometry (Thermo Q-Exactive). Lower limits of quantification were 0.1 ng/ml for simvastatin, 0.5 ng/ml for curcumin, and 1.0 ng/ml for quercetin. Inter- and intra-day precision was less than 15% for all three analytes.

Microbiota Analysis

Fecal samples were collected at baseline, day 14 and day 29 using standardized collection kits and stored within 2 hours at -80°C. QIAamp PowerFecal DNA Kit (Qiagen, Hilden, Germany) was used for DNA extraction. V3-V4 region of the 16S rRNA gene was amplified and Illumina MiSeq platform was used for sequencing. Bioinformatics analysis was performed using QIIME2 pipeline. SILVA database was used for taxonomic assignment. Alpha-diversity

(Shannon, Chao1) and beta-diversity (weighted UniFrac) values were recovered.

Metabolite Quantification

Short-chain fatty acids (acetate, propionate, butyrate) were quantified with gas chromatography-mass spectrometry. Bile salt hydrolase activity was measured by colorimetric assay with taurocholic acid as substrate. β-glucuronidase activity was measured using 4-nitrophenyl-β-D-glucuronide as substrate, and enzyme activity was expressed in units/g of feces.

Statistical Analysis

Sample size for detecting a 1.5-fold difference in AUC with 80% power and α=0.05 was determined. Pharmacokinetic parameters were calculated using non-compartmental analysis (Phoenix WinNonlin 8.3). Groups were compared using ANOVA followed by post-hoc Tukey's test. Microbiota were analyzed by permutational multivariate ANOVA (PERMANOVA) and linear discriminant analysis. Correlation was evaluated using Spearman's rank correlation. Statistical significance was at $p<0.05$.

4. Results

Table 1: Baseline Characteristics and Microbiota Composition

Parameter	Control (n=30)	Probiotic (n=30)	Prebiotic (n=30)	Synbiotic (n=30)	p-value
Demographics					
Age (years)	28.4 ± 6.2	29.1 ± 5.8	27.9 ± 6.5	28.7 ± 6.0	0.742
Gender (M/F)	15/15	14/16	16/14	15/15	0.891
BMI (kg/m²)	23.2 ± 2.8	23.6 ± 3.1	22.9 ± 2.9	23.4 ± 2.7	0.656
Baseline Microbiota Diversity					
Shannon Index	3.42 ± 0.68	3.38 ± 0.72	3.45 ± 0.65	3.41 ± 0.69	0.923
Chao1 Index	284.6 ± 45.2	278.9 ± 48.7	289.3 ± 42.8	281.4 ± 46.9	0.678
Key Bacterial Phyla (%)					
Bacteroidetes	42.8 ± 8.9	41.6 ± 9.4	43.2 ± 8.5	42.1 ± 9.1	0.834
Firmicutes	48.2 ± 9.6	49.1 ± 8.8	47.9 ± 10.2	48.6 ± 9.3	0.912
Actinobacteria	4.1 ± 1.8	4.3 ± 1.9	4.0 ± 1.7	4.2 ± 1.8	0.845
Proteobacteria	3.9 ± 2.1	3.8 ± 2.0	4.1 ± 2.3	3.9 ± 2.2	0.897

Table 2: Pharmacokinetic Parameters Before and After Intervention

Drug	Parameter	Control	Probiotic	Prebiotic
Cmax (ng/mL)	Pre: 12.4 ± 3.2 Post: 13.1 ± 3.8	Pre: 12.8 ± 3.5 Post: 18.6 ± 4.2***	Pre: 12.2 ± 3.1 Post: 14.3 ± 3.9	Pre: 12.6 ± 3.4 Post: 28.9 ± 5.7***
$AUC_{0–24}$ (ng·h/mL)	Pre: 89.6 ± 18.4 Post: 92.3 ± 20.1	Pre: 91.2 ± 19.8 Post: 152.4 ± 28.9***	Pre: 88.7 ± 17.9 Post: 108.6 ± 23.2	Pre: 90.1 ± 18.9 Post: 207.3 ± 32.4***
Tmax (h)	Pre: 2.1 ± 0.5 Post: 2.0 ± 0.6	Pre: 2.2 ± 0.4 Post: 1.8 ± 0.4*	Pre: 2.1 ± 0.5 Post: 1.9 ± 0.5	Pre: 2.0 ± 0.4 Post: 1.6 ± 0.3**
Cmax (ng/mL)	Pre: 8.9 ± 2.6 Post: 9.2 ± 2.8	Pre: 9.1 ± 2.7 Post: 13.7 ± 3.4**	Pre: 8.7 ± 2.5 Post: 10.4 ± 3.1	Pre: 9.0 ± 2.6 Post: 16.2 ± 3.9***
$AUC_{0–24}$ (ng·h/mL)	Pre: 41.8 ± 12.3 Post: 43.6 ± 13.1	Pre: 42.3 ± 11.9 Post: 70.2 ± 16.8***	Pre: 41.2 ± 12.6 Post: 51.9 ± 14.7	Pre: 41.9 ± 12.2 Post: 75.4 ± 17.3***
Tmax (h)	Pre: 3.2 ± 0.8 Post: 3.1 ± 0.9	Pre: 3.3 ± 0.7 Post: 2.6 ± 0.6**	Pre: 3.2 ± 0.8 Post: 2.9 ± 0.7	Pre: 3.1 ± 0.8 Post: 2.3 ± 0.5***
Cmax (ng/mL)	Pre: 15.6 ± 4.2 Post: 16.1 ± 4.6	Pre: 15.9 ± 4.1 Post: 21.8 ± 5.3**	Pre: 15.4 ± 4.0 Post: 17.9 ± 4.8	Pre: 15.7 ± 4.3 Post: 25.1 ± 6.2***

$AUC_{0–24}$ (ng·h/mL)	Pre: 124.8 ± 28.6 Post: 128.3 ± 30.2	Pre: 126.2 ± 29.1 Post: 178.4 ± 34.7**	Pre: 123.9 ± 28.9 Post: 145.7 ± 32.4	Pre: 125.6 ± 28.4 Post: 200.9 ± 38.1***
Tmax (h)	Pre: 2.8 ± 0.7 Post: 2.9 ± 0.8	Pre: 2.7 ± 0.6 Post: 2.2 ± 0.5*	Pre: 2.8 ± 0.7 Post: 2.5 ± 0.6	Pre: 2.9 ± 0.7 Post: 2.0 ± 0.4**

*p<0.05, **p<0.01, ***p<0.001 compared to pre-intervention values within the same group

Table 3: Microbiota Changes and Metabolite Production

Parameter	Control	Probiotic	Prebiotic	Synbiotic
Microbiota Diversity (Post-intervention)				
Shannon Index Change	+0.08 ± 0.31	+0.42 ± 0.28**	+0.29 ± 0.35*	+0.67 ± 0.31***
Chao1 Index Change	+8.2 ± 22.4	+38.6 ± 28.1**	+24.7 ± 26.8	+52.3 ± 29.7***
Key Species (% relative abundance)				
A. muciniphila	2.1 ± 1.2	3.8 ± 1.6**	2.9 ± 1.4	5.2 ± 1.8***
F. prausnitzii	8.4 ± 2.6	12.7 ± 3.1**	10.2 ± 2.9	15.8 ± 3.4***
L. rhamnosus	0.3 ± 0.2	2.1 ± 0.8***	0.4 ± 0.3	2.4 ± 0.9***
B. longum	1.8 ± 0.7	4.2 ± 1.3***	2.1 ± 0.8	4.8 ± 1.4***
Metabolites (μmol/g feces)				
Total SCFAs	78.6 ± 18.2	106.4 ± 22.7**	89.7 ± 20.1	124.8 ± 25.3***
Acetate	52.3 ± 12.4	68.9 ± 15.8**	58.1 ± 13.7	79.4 ± 17.2***
Propionate	18.7 ± 4.6	26.2 ± 6.1**	21.9 ± 5.2	31.6 ± 6.8***
Butyrate	7.6 ± 2.2	11.3 ± 2.8**	9.7 ± 2.7	13.8 ± 3.3***
Enzymatic Activity				
BSH activity (U/g)	12.4 ± 3.8	18.9 ± 4.6**	14.7 ± 4.2	22.6 ± 5.1***
β-glucuronidase (U/g)	8.9 ± 2.1	13.2 ± 3.4**	10.6 ± 2.8	15.7 ± 3.9***

*p<0.05, **p<0.01, ***p<0.001 compared to control group

Discussion

The outcomes of this study demonstrate that gut microbiota modulation directed towards an intended reaction is a new and promising approach to enhancing the oral bioavailability of drugs with low water solubility. The increased drug absorption observed, particularly with synbiotic treatment, represents powerful evidence for the therapeutic prospects of the method in pharma drug research and development as well as in individualized medicine. (Koppel et al., 2017).

Mechanisms of Enhanced Bioavailability

The increased effectiveness of synbiotic therapy over probiotic or prebiotic therapy alone suggests synergistic interaction between probiotic live bacteria and their preferred substrates. Various mechanisms can be responsible for the improvement of drug bioavailability noted. The increased activity of bile salt hydrolase (BSH) in intervention groups, particularly the synbiotic group, is supportive of enhanced deconjugation of bile acids. This process facilitates the increase in solubilization of poorly soluble drugs by increasing the size of the bile acid pool and promoting micelle formation necessary for lipophilic drug absorption. (Saad et al., 2012).

The major increase in short-chain fatty acid (SCFA) production, most notably butyrate, plays a role in drug absorption enhancement in several ways. SCFAs are the principal energy substrates of colonocytes and, further, stimulate intestinal epithelial cell proliferation and barrier function. SCFA production-induced intestinal acidification can also increase drug dissolution, with a particular effect on weakly basic drugs. The association of SCFA concentrations with improved bioavailability is in accordance with this mechanistic pathway. (Zimmermann et al., 2019)

The dramatic elevations in Akkermansia muciniphila concentrations between best response and intervention groups within the synbiotic group are fascinating. This mucolytic bacterium has been linked with increased intestinal barrier function and increased drug permeability. Our results point towards A. muciniphila as a novel predictive biomarker for subject response to microbiota-targeting bioavailability enhancement approaches.

Drug-Specific Reactions

Differential modulation of the three model drugs suggests the specificity of microbiota-mediated facilitation of bioavailability. The largest increase (2.3-fold increase by synbiotic treatment) was shown by the lipophilic statin simvastatin as predicted by its dependency on solubilization mediated by bile acid.The intermediate degree of enhancement observed with curcumin (1.8-fold increase) can be attributed to its susceptibility to phase II metabolism, which may be modulated by microbial metabolites. Quercetin's modest but significant enhancement (1.6-fold increase) suggests flavonoid compounds can be enhanced by microbiota modulation by several mechanisms, possibly through microbial β-glucosidase activity that hydrolyses quercetin glycosides to more bioavailable aglycones.

The lowered Tmax in all intervention groups suggests that microbiota modulation not only enhances the extent of absorption but also increases drug uptake rate. This finding has important clinical implications, as higher drug absorption rates may lead to more predictable therapeutic effects and reduced inter-individual variability. (Maier et al., 2021).

Clinical Implications and Translational Potential

The degree of bioavailability increases observed here has significant clinical significance. For simvastatin, the 2.3-fold increase in AUC can potentially allow for substantial reduction of dose with preservation of therapeutic effect and thereby prevent adverse effects such as myopathy due to overdosing. Similarly, enhanced bioavailability of curcumin and quercetin may increase the therapeutic use of these compounds, whose preclinical efficacy has been diminished by their poor oral absorption. (Olle, 2013).

The interindividual variation observed in response to microbiota modulation, as manifested in the correlation with baseline microbiota composition and the improvement in bioavailability, suggests potential for individualized approaches. The subjects who initially experienced lower microbial diversity showed greater improvements, suggesting that interventions involving microbiota may be particularly beneficial in patients with dysbiotic gut microbiota.

Limitations and Future Directions

There are some limitations of this study that must be highlighted. The comparatively short intervention period (28 days) might not capture the peak effect of microbiota modulation because there could be some long-term positive effects which take more time to manifest. The research was also conducted in healthy volunteers, and the result in patient populations with deranged gut function or an interrupted microbiota because of disease or intake of medicines could be varied.

Mechanisms of observed benefit must be explored more with mechanistic studies. Future studies must determine which strains of bacteria and metabolites are responsible for enhanced drug absorption and design targeted interventions based on individual microbiota profiles. Long-term safety studies must also determine sustained effects of chronic microbiota modulation. (Mallick et al., 2019)

Regulatory and Development Considerations

The integration of microbiota modulation strategies into drug development is difficult but also rich in opportunity. Regulation for combination products that incorporate drugs and microbiota-modulating factors is evolving and will be crucial to standardize probiotic and prebiotic products for reproducible clinical response. Companion diagnostics to identify patients that will benefit most from microbiota-enhanced bioavailability is a rich area of individualized medicine.

Future Directions

Future research avenues include the formulation of personalized strategies for microbiota modulation based on the patient's microbiota profile. Sophisticated computational models capable of forecasting the interaction between drugs and microbiota may also optimize treatment.

Additionally, design of engineered bacterial strains with enhanced drug-metabolizing capacity may provide more predictable and controllable therapeutic interventions.

The integration of pharmacomicrobiomics into pharmaceutical development pipelines could lead to improved and safer medicines (Haiser & Turnbaugh, 2012). It would involve the testing of drug candidates for susceptibility to microbial metabolism and formulation development to modulate these interactions for optimization.

Future research should focus on developing standardized protocols for microbiota modulation, constructing safety profiles for microbial therapeutics, and creating predictive models for drug-microbiota interactions. This discipline will further be advanced by future collaborative work among microbiologists, pharmacologists, and clinicians, transforming research knowledge into clinical benefit.

Conclusion

This work provides compelling evidence that certain gut microbiota modulation, such as symbiotic intervention, can significantly enhance the oral bioavailability of poorly soluble drugs. These benefits appear to be mediated by a number of different mechanisms, including enhanced bile acid metabolism, increased SCFA production, and improved intestinal barrier function. These findings hold new avenues for treating one of the most recalcitrant issues in drug discovery and show that the gut microbiota has to be considered as a modifiable variable for optimal drug absorption. The prospect of individualized treatment directed towards a person's microbiome signature is a paradigm shift towards precision medicine approaches in pharmaceutical treatment.

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