Gut-Brain Signaling in Response to Intestinal Microbial Dysbiosis: Patient-Derived Enteroids and Colonoids in Parkinson's Disease

Richard A. Manfready, MD, AM[1]; Maliha W. Shaikh, MS[1]; Lijuan Zhang, MD[1]; Kristi M. Lawrence[1]; Robin M. Voigt, PhD[1]; Christopher B. Forsyth, PhD[1]; Christopher G. Goetz, MD[2]; Ali Keshavarzian, MD[1]

[1]Department of Internal Medicine, Division of Digestive Diseases and Nutrition; [2]Department of Neurological Sciences; Rush University Medical Center, Chicago, IL, United States

Objectives

To evaluate the impacts of intestinal dysbiosis on enteroendocrine function in Parkinson's disease (PD) by:

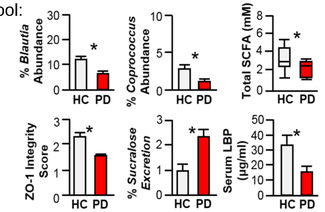
1) Detecting patterns of microbial dysbiosis in PD,
2) Determining the contribution of intestinal dysbiosis to L-cell function,
3) Constructing *in vitro* organoid models from patient-derived tissue to simulate the effects of microbial ecosystems and their metabolites on enteroendocrine function.

Background

- PD patients are known to have significantly disrupted intestinal microbiota [1], but the functional significance of this dysbiosis remains elusive.
- The relative dysbiosis of PD favors pro-inflammatory bacteria at the expense of short-chain fatty acid (SCFA)-producing species [2-4]. These findings may substantiate observations that constipation and other GI symptoms such as bloating predate motor symptoms, and that α-synuclein aggregates are found in gut epithelial tissue prior to brain involvement.
- We have determined the impact of the pro-inflammatory milieu on SCFA, inflammatory markers, and glucagon-like peptide-1 (GLP-1), an incretin hormone with neuroprotective effects [5].
- We have engineered a 3-dimensional organoid model from patient biopsies that can be used to investigate the impacts of stool microbiota and prebiotics on GLP-1 secretion, with the goal of maximizing secretion in the PD subject.

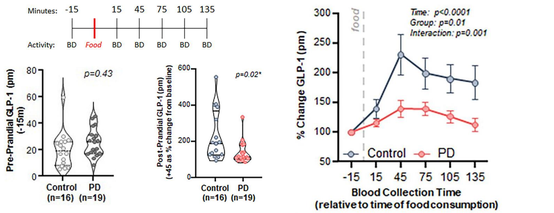
Microbial Dysbiosis in PD

- Our group and others have previously shown that microbial aberrations in the stool of PD subjects are characterized by two main patterns relative to healthy control stool:
  - 1) Increased relative abundance of "pro-inflammatory" bacteria associated with pathogen-associated molecular patterns such as lipopolysaccharide, which trigger inflammatory responses signaling production of inflammatory cytokines such as TNF-α, IL-8, IL-1, and interferon-γ. Such bacteria include those in the family Enterobacter and the genus *Lactobacillus*.
  - 2) Decreased relative abundance of "anti-inflammatory" bacteria that produce SCFA, which have been found to be neuroprotective in models of PD. Such bacteria include *Bifidobacteria* and *Blautia.*
  - The PD gut is also characterized by impaired intestinal barrier integrity, as demonstrated by decreased tight junction protein ZO-1 and increased sucralose excretion in the urine. (Adapted from Keshavarzian, et al., 2015)

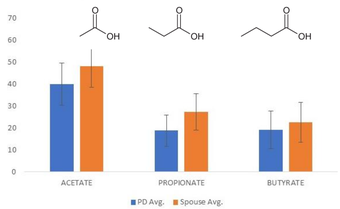


SCFA and GLP-1 in PD

- GLP-1 secretion from enteroendocrine cells is triggered by the interaction of SCFA with luminal receptors. GLP-1 response to a meal is typically bimodal with the greatest peak 45 minutes postprandially.
- We demonstrated that the postprandial GLP-1 response is attenuated in PD patients relative to household-matched healthy controls who consume a standardized meal and undergo pre- and postprandial GLP-1 measurement by ELISA. [6]



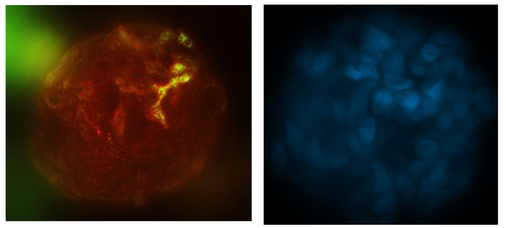
- Metabolomics analysis revealed lower concentrations of SCFAs in PD stool relative to control stool (in mM).
- These findings have produced a model whereby intestinal microbes signal through SCFAs and L-cells to secrete GLP-1. [7]
- Our work has demonstrated that, in humans, prebiotic fiber consumption improves microbial species biodiversity in the gut, increases systemic SCFA, and improves UPDRS motor scores. [8]
- Yet, it remains unknown whether this process is attributable directly to GLP-1 secretion, and why SCFA-GLP-1 regulation is altered in PD. It is hypothesized that L-cell number, L-cell functional capacity, intestinal SCFA content, or a combination of these factors may be defective in PD. We propose an *in vitro* model to test these hypotheses.



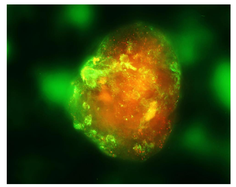
	Control	PD	Test
Number	16	19	n/a
Sex (*n*, %)			
Male	4 (25%)	13 (68%)	$p = 0.03^{\ddagger}$
Female	11 (69%)	6 (32%)	
Not reported	1 (6%)	0 (0%)	
Age (years)			
Average	66.4	66.8	$p = 0.87^{\dagger}$
Range	56–80	55–81	
Race (*n*,%)			
Caucasian	13 (81%)	18 (95%)	$p = 0.28^{\ddagger}$
African-American	1 (6%)	1 (5%)	
Not reported	2 (13%)	0 (0%)	
BMI			
Average	26.8	28.0	$p = 0.68^{\dagger}$
Range	20–35	20–48	
Age at PD onset (years)			
Average	n/a	56.1	n/a
Range	n/a	45–74	
Disease duration (years)			
Average	n/a	11.4	n/a
Range	n/a	7–18	
MDS-UPDRS			
Average	n/a	17.0	n/a
Range	n/a	0–37	
H&Y			
Median	n/a	2	n/a
Range	n/a	2–3	
Medication (*n*,%)			
Dopamine precursor	–	19 (100%)	
Dopamine agonists	–	10 (53%)	n/a
Glutamate antagonist	–	8 (42%)	
Anticholinergics	–	2 (11%)	
COMT inhibitors	–	4 (21%)	
MAO-B inhibitors	–	7 (37%)	
Antidepressant	–	1 (5%)	

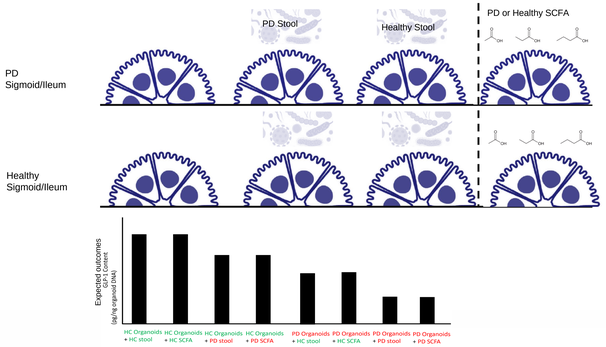
‡*Chi square analysis.*
†*Student's t-test.*

Enteroids and Colonoids



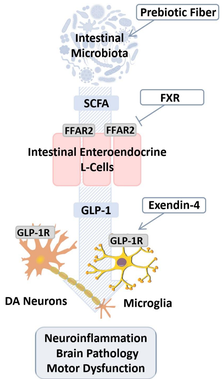
- 3D basal-out organoids were developed from ileum and sigmoid of PD patients and healthy controls [9] and embedded in matrix scaffold.
- A low-retention surface technique was used to create apical-out organoids so the luminal side is exposed to coculture media. [10]
- The organoids were treated with stool or pure SCFA combinations (from PD patients or healthy controls).
- ELISA was used to measure GLP-1 secretion.
- Goal is to quantify GLP-1 secretion by PD or healthy organoids in response to PD or healthy stool, and then determine phenotypic differences between PD and healthy L-cells.
- The organoids were stained for chromogranin-C (green) and GLP-1 (red) to identify L-cells (above, left). These organoids were stored in a repository for future stool/SCFA co-culture studies, as well as studies of L-cell density in PD. DAPI staining (above, right).
- Organoids survive co-culture with stool and pure SCFA dilutions.





Discussion and Future Directions

- We have successfully modeled the human intestinal enteroendocrine system in Parkinson's disease using patient-derived enteroids and colonoids that can be co-cultured with stool and short-chain fatty acids, with the goal of measuring GLP-1 secretion by L-cells.
- These organoids recapitulate L-cell phenotypes as determined by human intestinal stem cells.
- Our previous work has shown that postprandial GLP-1 (which is neuroprotective in several important PD models) is diminished in patients with PD. We have also shown that PD patients have gut microbiota that favor less production of SCFAs, which normally act directly on enteroendocrine cells to promote GLP-1 secretion.
- The organoid model is poised to probe the mechanism of GLP-1 secretion in response to stool or SCFA.
- The model is also a platform technology from which an array of microbiota-gut interactions can be queried using epithelium derived from patient stem cells.



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