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Relationship Between The Gut-Brain Axis, Parkinson's Disease, and Use of Canines as Animal Models in Neurodegenerative Disease Research.

Kimberly Dao
Iowa State University

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Kimberly A. Dao
Iowa State University
Masters of Biomedical Sciences

Abstract

Parkinson's disease (PD) is known to develop when there are decreased dopamine levels in the substantia nigra and development of alpha-synuclein neurons in the brain. However, recent studies have suggested that the gut-brain axis plays a large role in the development of PD. As a person ages, the cellular defense mechanisms in the gastrointestinal tract weakens, causing changes in the gut microbiota. This can eventually lead to changes such as dysbiosis, increased oxidative stress, and eventually alpha-synuclein neuron formation in the enteric nervous system in those who are susceptible to PD. The alpha-synuclein neurons spread to regions of the brain via the gut-brain axis. These changes along with other environmental factors can assist in the development of PD. Current L-DOPA treatment for PD are highly effective at minimizing symptoms, but can leave patients experiencing debilitating side effects during the "off" state when plasma L-DOPA levels are low. Researchers have been in search to improve treatment by exploring gastroretentive L-DOPA treatment and use of probiotics. Additionally, canines should be used as animal models for neurodegenerative disease research due to their various similarities to humans. Canines specifically are similar in their diets, digestive tract, aging process, and cerebral anatomy.

Keywords: Parkinson's disease, gut-brain axis, dopamine, alpha-synuclein neurons, oxidative stress, dysbiosis, gut microbiota, L-DOPA, probiotics, canine, animal models

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disease in the United States.[1] It is estimated that by 2050, approximately 1.34 million people will be affected with PD in the United States.[1] PD occurs when there is a decrease in active dopaminergic neurons in the substantia nigra, which leads to a decline in dopamine levels. Patients with PD also have been seen to possess misfolded alpha-synuclein neurons in the brain. This causes the impairment of controlled voluntary movement, [1,2,3]

There are various symptoms that accompany PD. However, the main symptoms are split up into motor deficits and non-motor deficits. Motor deficits include muscle rigidity, resting tremor, and gait disturbances. Non-motor deficits tend to include decrease sense of smell, dementia, depression, and gastrointestinal (GI) disorders.[4]

There is currently no "cure" for PD. Current treatment options for PD are geared towards restoring dopaminergic transmission. This helps improve symptoms, but does not affect the neurodegenerative process.[1] D2-like agonist can be used to increase dopaminergic signaling in the striatum. These have been seen to decrease motor deficits. There are also pharmaceutical treatments available that inhibit enzymes that catabolize dopamine, which can help increases availability of endogenous dopamine.[1,5] Currently, the most common treatment for PD is levodopa (L-DOPA), which is a precursor for dopamine. PD patients typically take L-DOPA orally several times per day and a peripheral decarboxylase inhibitor is most often used in combination. This allows the majority of treatment to reach the central nervous system (CNS) by inhibiting the conversion of L-DOPA to dopamine peripherally.[6] L-DOPA is currently the most successful treatment for PD. However, many patients start to experience significant side effects with long-term use. These side effects include dyskinesia, hallucinations, increased cognitive impairment, depression, anxiety, and sleep disorders. [6] The "on-off" phenomenon refers to the pharmacokinetic effects of current L-DOPA treatment. Patients who take L-DOPA experience a rise and fall of plasma L-DOPA levels. When plasma L-DOPA levels are high, patients are seen to have fewer side effects. When plasma L-DOPA levels are lower, patient tend to experience dyskinesia, cognitive impairment, and other side effects. [7] This has been a large topic of discussion in PD

research. Researchers are interested in finding a noninvasive pharmaceutical treatment that can consistently keep plasma L-DOPA levels at a desired level.

The purpose of this literature review is to explore the connections between PD and the gut-brain axis. This review will also discuss alternative options to treatment, including gastroretentive L-DOPA treatment and probiotic use. Lastly, this review will discuss the use of canines as animal research models.

Gut Microbiota and The Gut-Brain Axis

Gut microbiota refers to the various bacterial species that reside in the gastrointestinal tract.[8] The bacterial community is diverse and each division has their own role. Bacteria in the gastrointestinal tract mainly consists of phyla Firmicutes and phyla Bacteriodietes.[4,9,10,11] These species are referred to as beneficial flora and have many beneficial roles in regulating immunity and nutrition. Beneficial flora have the ability to ferment carbohydrates, produce short-chain fatty acids, metabolize various substances such as drugs, and protect the gastrointestinal tract from various pathogens.[4] In contrast, opportunistic bacteria also reside in the gut and have the ability to cause infection. Gastrointestinal disorders can begin to arise when dysbiosis occurs, which is characterized by an imbalance in beneficial flora and opportunistic bacteria.[4]

The gut-brain axis refers to the intimate relationship between the gastrointestinal tract and the brain. Recent studies have revealed an intricate bidirectional neuroendocrine system that allows both systems to communicate with each other.[8] The main contributors to the gut-brain axis involve the enteric nervous system (ENS), the central nervous system (CNS), and the gut microbiota.[8] These systems essentially come together to keep the gut and the mind healthy and at equilibrium. Disruption of this system have been suggested to cause various diseases, such as irritable bowel syndrome, anxiety, depression, dementia, and PD.[12]

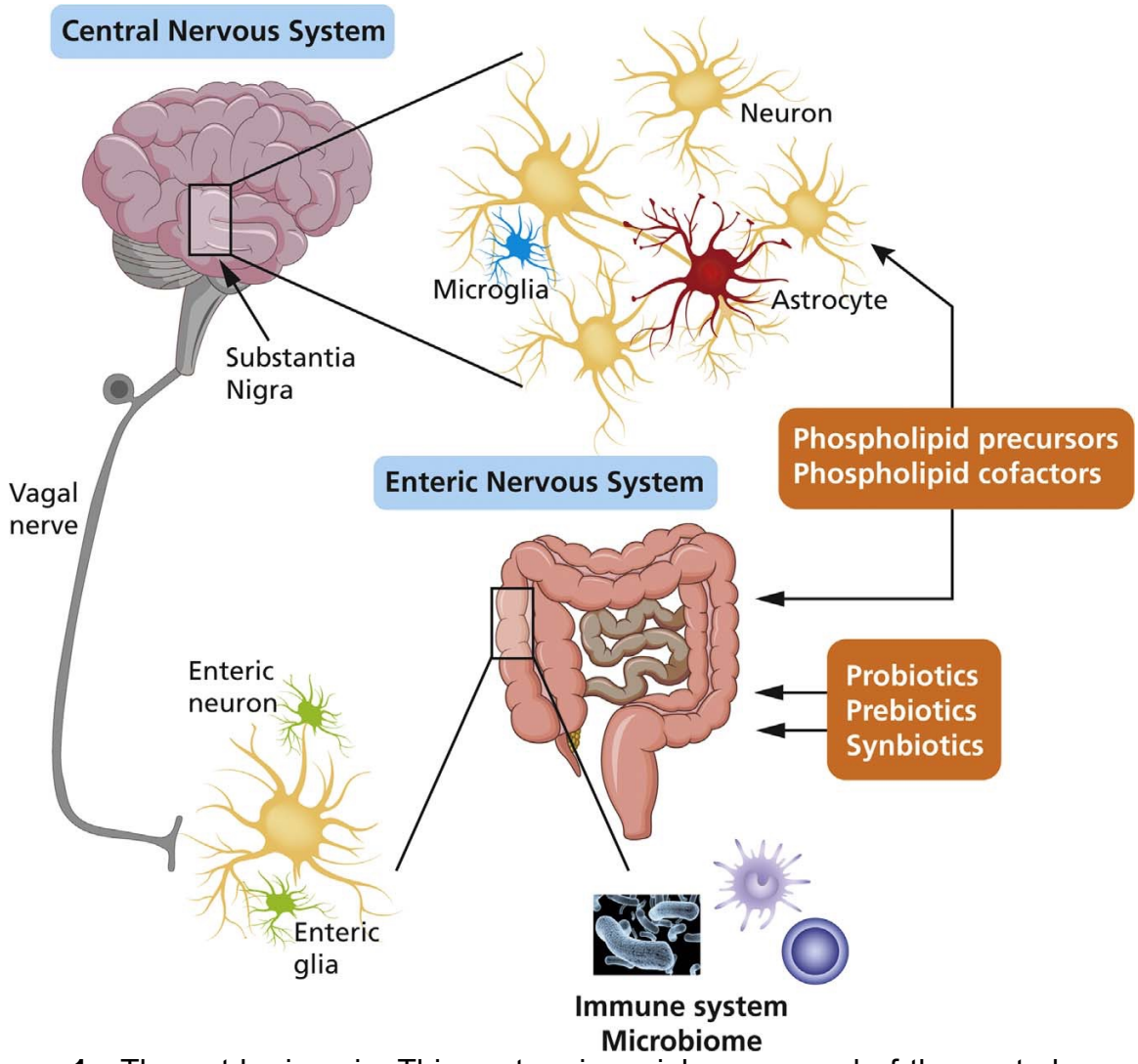

Figure 1. The gut-brain axis. This system is mainly composed of the central nervous system, enteric nervous system, and gut microbiota. Neuronal membrane formation can be influenced by dietary phospholipid precursors and cofactors, reducing inflammation. Probiotics, prebiotics, and synbiotics can also improve gut health by improving the composition of the gut microbiota.[13]

Relationship of Parkinson's Disease and the Gut-Brain Axis

There are many factors that contribute to the relationship between PD and the gut-brain axis. PD has been linked environmental stressors, diet, and emotional stress. Environmental toxins, such as pesticides, can be found in the food humans consume and the environment we surround ourselves in.[8,14] Age is a large factor in abnormal gut function. As we age, the cellular defense mechanisms in the gut weaken and damage is

more likely to be occur as the body is exposed to opportunistic bacteria and stressors.[8,15] These various stressors can eventually cause disruption in the gut microbiota. Proportions of phyla Bacteriodetes and Firmicutes also fluctuate with age.[8] When dysbiosis occurs within the gut, the imbalance can result various gut dysfunction processes. This can lead to changes in the gut microbiota, oxidative stress and chronic low-grade inflammation.[8,16] Additionally, accumulation of alpha-synuclein neurons in the gastrointestinal tract has been discovered in patients with PD.[8]

Dysbiosis occurring within the gut can lead to many changes in the gut microbiota. With constant dysbiosis, these changes can become chronic and lead to long-term complications. A study including 72 patients diagnosed with PD and 72 healthy control patients showed that there was a decreased amount of Prevotellaceace species in the feces of those who were diagnosed with PD compared to the healthy control patients. Prevotellaceace is a main producer of mucin, which is a protein that is highly glycosylated and provides protective barrier along the walls of the gut, preventing pathogens from entering. Additionally, those who experienced postural instability and gait difficulty were seen to have more Enterobacteriacease in their feces. Enterobacteriacease has been seen in other feces of other neurodevelopment disorders as well, such as autism. It has been suggested that Enterobacteriacease is seen in more severe cases of PD and are questioning if it has an endotoxin-induce cascade.[17] In another study involving 38 patients affected by PD and 34 healthy controls, fecal samples and intestinal biopsies were taken from the sigmoid mucosa. Results showed a significant decrease in anti-inflammatory butyrate producing bacteria (genera *Blautia, Coprococcus,* and *Roseburia)* in patients with PD. Additionally, an increase in proinflammatory Proteobacteria of genus *Ralstonia* was seen in PD patients.[18] It is shown that dysbiosis can lead to drastic changes in the gut microbiota that can contribute gastrointestinal issues and various symptoms that patients with PD may experience. In another study performed by Sampson et al. 2016, fecal microbiota from human patients diagnosed with PD was transferred to mice in order to identify if the gut microbiota would persist and affect disease outcomes. Germ free mice that were given human PD patient's feces were referred to "humanized" mice. The control germ free mice were given feces from healthy patients. Results revealed that humanized mice

displayed significantly similar fecal microbiota to human patients with PD. The control group displayed larger differences in fecal microbiota than the healthy humans.[19] This concluded that the differences in fecal microbiota remained persistent when transferred over to another organism.

Oxidative stress has a large role in PD and can arise from various factors. Oxidative stress can occur when reactive oxidative species are overproduced. Reactive oxidative species (ROS) are produce in the mitochondria when electrons escape the electron transport chain. This mishap typically only occurs about 0.4-4% of time and a healthy host's and antioxidant defense mechanisms typically take care of them without causing harm.[8,20] However, with age related cellular degeneration and dysbiosis, this can cause various cellular and membrane damage that can eventually lead to cell death.[8,15] Prolonged excessive ROS can lead to modifications in proteins, nucleic acids, and lipids. While many neurons in the brain typically can withhold oxidative stress fairly well, there are some regions that are more vulnerable than others. A study reviewed by Wilde et al. 1997, Vornov et al. 1998, Sarnowska, 2002, and Wang et al. 2005 looked into oxidative stress in the hippocampus. The hippocampus was chosen because of the density of neurons involved in this area of the brain. The hippocampus was split up into 4 different regions, CA1 through CA4. Although regions CA1 and CA3 were composed in a similar structure containing pyramidal neurons, oxidative stress caused cell death to the pyramidal neurons of CA1. In contrast, most of the neurons in CA3 survived. This pattern of CA1 cell death to oxidative stress has specifically been seen in conditions, such as neurodegeneration and ischemia. [21] Oxidative stress can also change the metabolic and genomic expression of the microbiota population. These alterations can eventually cause physiological changes in the long run, changing how nutrients are metabolized and altering endocrine signaling.[8,14]

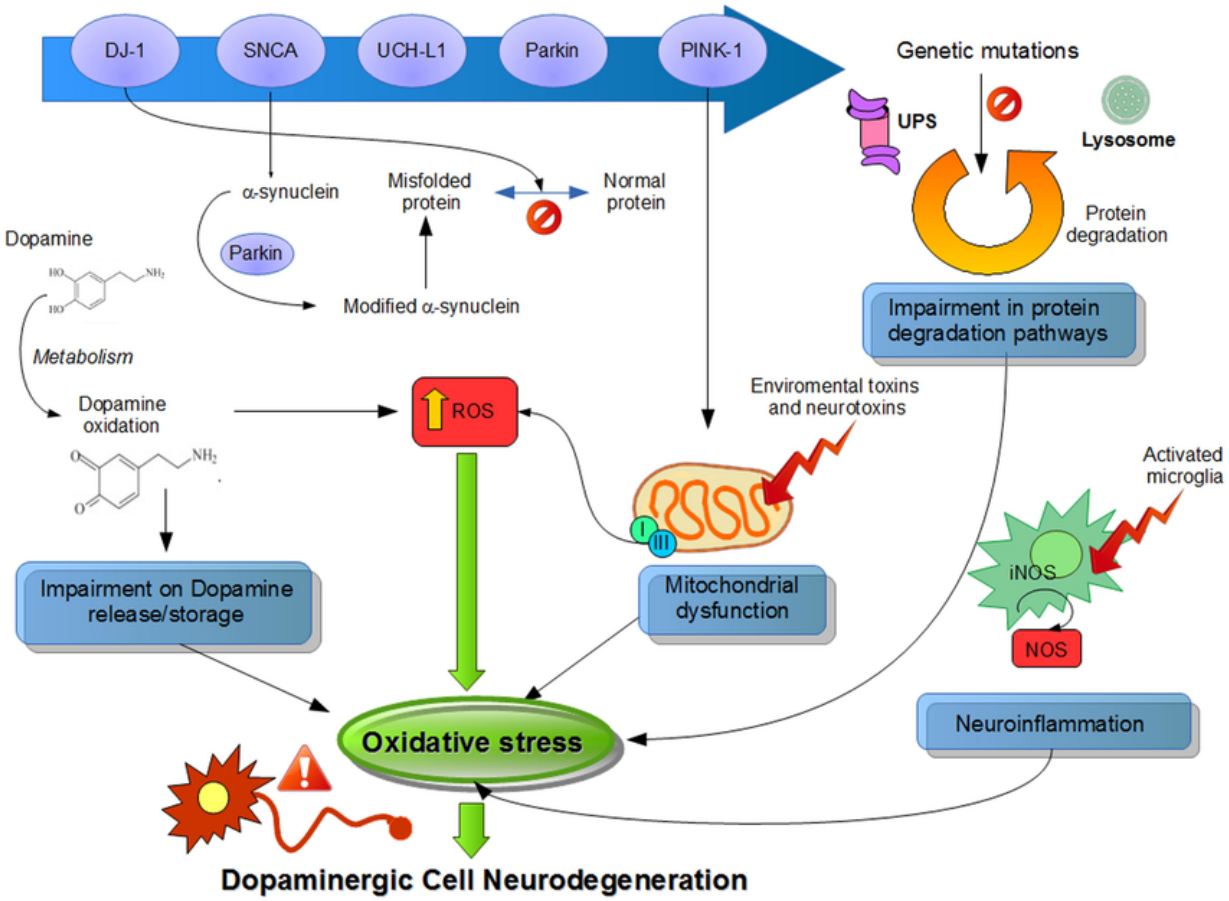

Figure 2. The development of oxidative stress in PD. Release of reactive oxidative species is the leading cause of oxidative stress. However, this process can progress from various processes, such as neuroinflammation, mitochondrial dysfunction, impairment of dopamine release and storage, and impaired protein degradation.[22]

Those affected with PD have been seen to experience chronic low-grade inflammation. When chronic inflammation is developed due to dysbiosis, it can lead to elevated cytokines and immune inflammatory cells that produce inflammatory mediators.[8] Chronic inflammation can also cause an increase in vascular permeability leading to edema, increased concentration of blood in gastrointestinal tract, tissue ischemia, hypoxia, and stasis.[23,24] Additionally, chronic inflammation can also lead to oxidative stress due to hypoxia. In order for the human body to adjust to this new environment, cell metabolism undergoes changes. These changes include delaying oxidative metabolism, conversion of xanthine dehydrogenase to oxidase, and depletion of adenosine triphosphate (ATP). Overall, these changes can also lead to reactive

oxygen intermediates (ROI) and this can either lead to oxygen reaching the cells but not being entirely reduced, or neutrophils reaching the inflamed portion and effecting the anaerobic glycolysis process, releasing ROI as a by-product. Changes in energy metabolism and disturbances in oxygen handling also occur when immune cells are activated. Oxidative phosphorylation is affected when macrophages fluctuate inflammatory activity. Chronic inflammation causes these symptoms to constantly be activated, allowing the persistence of macrophages to effect anaerobic glycolysis, leading to oxidative stress. Overall, the body adapts with chronic shortages and redisposition of resources during chronic inflammation. There is a constant fluctuation between energy storage and energy consumption by organs, which leads to competition for nutrients.[24]

Accumulations of alpha-synuclein neurons have also been discovered in patients with PD and have been one of the largest topics of discussion. In a study performed by Shannon, Keshavarzian, Dodiya, Jakate, and Kordower, 2012, three old sigmoid mucosa biopsies were obtained from patients with known PD and analyzed for alpha-synuclein by immunostaining. When compared to 23 healthy controls, it was found that all three samples from patients with known PD displayed alpha-synuclein pathology.[25] It was also discussed that the samples were obtained 2-5 years prior to when patients initially showed motor deficits.[25] This suggests that alpha-synuclein neurons form prior to development of neurological symptoms. The combination of low-grade inflammation and oxidative stress, alpha-synuclein develops in a similar pattern to prions.[8] It is hypothesized that alpha-synuclein is developed in the gut over time due to enteroendocrine cells (EECs) taking up toxins from the basolateral surface. As more toxins are taken up over a long period of time, there is an abnormal accumulation of aggregated alpha-synuclein protein in the enteric nervous system, as seen in Figure 3 .[23,19] These alpha-synuclein proteins that develop in the gut are hypothesized to spread to the brain through the vagus nerve.[19,26] Interestingly, it is seen that non-tremor dominant phenotype have been seen to have more severe alpha-synuclein pathology in the ENS. It has been suggested that this is linked to the higher amount of Enterobacteriacease.[17] The accumulation of alpha-synuclein neurons has been one of

the main neurological hallmarks for PD. The presence of them in the gut emphasizes the connection between PD and the gut-brain axis.

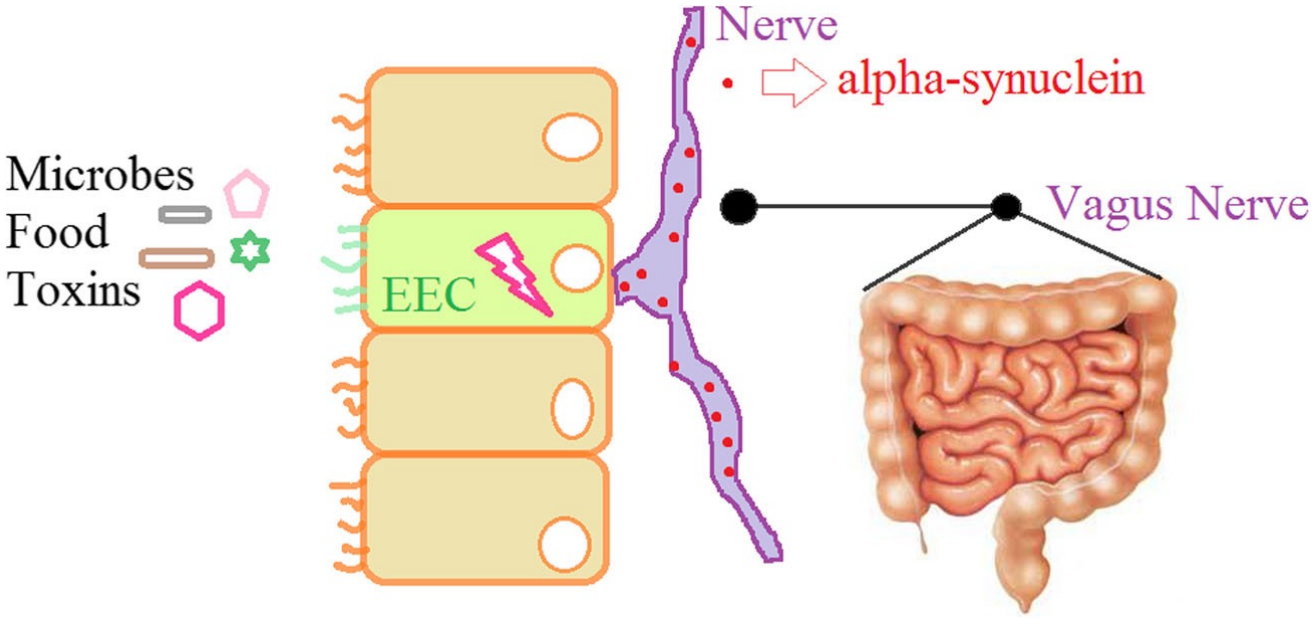

Figure 1. Enteroendocrine cells (EECs) are exposed to the lumen and in contact with various microbes, food, and toxins produced by gut microbes. The enteric nerve and glia is in contact with the basolateral surface of EECs. Aggregation of alpha-synucleins can occur depending on the toxins taken up by EEC. The aggregated proteins can migraine to enteric nerves, causing a pathogenic cascade. This can lead to abnormal accumulation of alpha-synuclein proteins in the gut.[23]

A study discussed by Sampson et al. 2016 was performed involving alpha-synuclein overexpressing (ASO) mice. The experimental group of ASO mice was raised to have complex gut microbiota (SPF-ASO). The control groups were wild type ASO mice. There were four series of tests performed to assess motor function. The beam traversal and pole descent tests measured gross motor control. The nasal adhesive removal test measured fine motor control. Lastly, the hindlimb clasping reflex test assessed striatal dysfunction. Results showed that the SPF-ASO group took a longer time to complete the beam traversal and pole descent tests. Then nasal adhesive removal test was also took a significant amount longer in SPF-ASO mice. Lastly, the hindlimb clasping reflex test was impaired in SPF-ASO mice. To examine if gut microbiota contributed to these results, the researchers performed an additional study

that reestablished ASO mice (GF-ASO) and wild-type mice (GF-WT) in a germ-free environment. Both groups underwent the 4 series of motor tests and results showed a reduction in motor deficits in ASO mice. Additionally, researchers noticed that SPF-ASO mice experienced less fecal output than GF-ASO. The fecal output of SPF-ASO mice also contained less water than GF-ASO. Fecal output of GF-ASO was not changed compared to the GF-WT mice.[19] This data indicates that gut microbiota has an effect on motor deficits and GI dysfunction. After performing immunofluorescence microscopy, Western blot, and dot blot analysis, the researchers reported a correlation between alpha-synuclein development in the CNS and gut microbiota. The GF-ASO mice displayed less alpha-synuclein development in the brain than the SPF-ASO mice.[19]

L-DOPA on Gut

Dopamine is a known enteric neurotransmitter and is highly concentrated in the gastrointestinal tract. It has been seen that blocking dopamine receptors causes a decline in the gastroprotective qualities dopamine offers. Additionally, blocking alpha-2 adrenoreceptors has also been seen to inhibit actions of dopamine and gastroprotection. There are many studies that have emphasized the connection between L-DOPA and the gastrointestinal tract. Some studies suggest that dopamine agonist have been discovered to prevent gastric injury induced by stress, ethanol, or medications.[16] It has also been seen that dopamine agonist help prevent cysteamine-induced duodenal ulcers.[16,27] In contrast, dopamine antagonist increases cysteamine-induced duodenal ulcers and has also been shown to enhance gastric lesions. [16] These effects indicate that endogenous dopamine might have an effect on gut health. A study was performed in 1996 investigating the effects of L-DOPA on gastric injury from ethanol. L-DOPA was given intraperitoneally to female Sprague-Dawley rats who had known gastric injury from ethanol. It was shown that L-DOPA was able to prevent further macroscopic lesions from developing. Additionally, the L-DOPA treatment was able to provide significant preservation of the gastric epithelium. [16]

Another study performed in 2012 investigated the delivery methods of L-DOPA using 3 different extended release (ER) tablets in patients with idiopathic PD. They compared the conventional ER tablet taken 3 times daily, a L-DOPA/carbidopa

gastroretentive ER formulations taken 2 times daily, and an immediate release (IR) formulation with IR/ER and without ER. Results indicated that the gastroretentive option achieved a constant L-DOPA concentration in the plasma, which reduced side effects associated with the fluctuation of plasma L-DOPA concentrations.[28] Using these results, researchers can consider developing gastroretentive L-DOPA treatment to avoid the "on-off" side effects caused by current L-DOPA treatment.

Prevention of Gut Dysfunction Using Probiotics

Many PD patients have seen benefits from using probiotics to help improve their sensory responses and decrease gut inflammation, which overall improves motility.[29] Those who experience decline in nutrition may also benefit from prebiotic and probiotic therapy, since these are seen to help with absorption of nutrients.[29]

As stated before, age is an important factor in the progression of neurodegenerative diseases. Probiotics have been seen to help decrease some of the effects of aging that are known to assist in neurodegeneration, which include oxidative stress, cell apoptosis, and decrease neurotransmitters.[8] Probiotics also help modulate neurological signaling in the gut, which overall improves inflammatory markers and reduces chronic inflammation.[8] Some studies have also suggested that taking probiotics may help the integrity of the intestinal tract with the blood brain barrier.[4] Additionally, some studies suggest that probiotics containing Lactobacilli and Bifidobacteria can actually reverse some side effects associated with PD. Probiotics that contain fermented milk contain Lactobacilli, which have been shown to decrease the amount of fecal Staphylococcus in patients with PD. The decrease in Staphylococcus has been seen to improve bowel movements. Bacterium Bacillus probiotics also have been suggested to convert L-tyrosine to L-DOPA. This is then converted to dopamine once DOPA decarboxylase is present.[4] Overall, this suggest that the use of probiotics might be more beneficial than keeping the gut healthy, but also preventing the development of gut dysfunction in patient with PD.

Relevance of Canine Model for Research

Animal models are used in research to potentially help translate data to develop pharmaceuticals and treatments for humans. Rodents have traditionally been the animal models for various research studies, including neurodegenerative research. As more research is being performed over time, studies are revealing that there are multiple limitations to using rodents as animal models. A large limitation in neurodegenerative disease research is that there is a limited amount of animal species that mimic a similar gut-brain axis system as humans. However, as of recent there has been more research suggesting canines as an ideal animal model, especially for neurodegenerative and gastrointestinal research.[30] This review will discuss the advantages of canines due to diet, digestive tract, aging process, and cerebral anatomy.

Humans and rodents have vastly different diets. Rodents typically consume a less complex diet than humans, which causes mice gut microbiota to be drastically different.[30,31] Mice gut microbiota can produce enzymes that they do not typically consume, such as ascorbic acid.[30,32] In contrast, humans consume a variety of different nutrients and do not synthesize the same enzymes as rodents do. Canines display a diet that is more closely related to humans and can adapt well to changes in diet.[30] Therefore, the gut microbiota in dogs may resemble humans more closely. Due to decades of domestication, canines also naturally live in an environment that most closely relates to a human's environment. Canines deal with similar environmental factors to humans, in comparison to the mice.[30,33] In addition, canines naturally have a diseased gastrointestinal tract when comparing to humans. This makes them good animal models for GI dysfunctions and neurodegenerative disorders. Therefore, canines are good disease models for diseases such as PD, Alzheimer's disease (AD), and irritable bowel syndrome.[30, 33, 34]

Additionally, the aging process of rodents is not as parallel to humans as canines. Studies have shown that at age 5-9 years old, canines display similar cognitive aging process to middle aged humans between 40-60 years of age. Additionally, some species of canines experience canine multiple system degeneration (CMSD), which is an inheritable movement disorder. Dogs that have CMSD experience impaired voluntary movements due to degeneration to multiple brain regions, including the substantia

nigra, caudate nucleus, and cerebellum.[30] Canines and humans also have similar brain structure and are described to have a gyrencephalic brain. In contrast, mice have a lissencephalic brain.[30] These points emphasize the benefits of using canines for neurodegenerative movement disorders, such as PD.

Conclusion

These findings help conclude that the cause of PD is multifactorial and cannot be pinpointed to one event. This review discusses how gut microbiota and the gut-brain axis influence the development of PD. The development of PD is suggested to stem from gut dysfunction years before symptoms develop. However, it is seen that the development of dysbiosis is caused by lifetime environmental factors.

Although L-DOPA is currently the main treatment for PD, researchers and pharmaceutical companies are continuously working to improve PD treatment. This review suggests that continuous plasma L-DOPA levels are important to limiting serious side effects. Gastroretentive L-DOPA treatments and probiotics have been suggested to help improve treatment and equilibrium of gut microbiota.

Lastly, this review discussed the benefits of using canines as animal models for research. Pertaining to neurodegenerative diseases and PD research, canines are suggested as the ideal animal model because of their gastrointestinal and cognitive similarities to humans.

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