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LEADING ARTICLE

Probiotics for Parkinson's disease: Current evidence and future directions

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Abstract

The gut–brain axis is a hot topic in Parkinson's disease (PD). It has been postulated that gut pathogens and dysbiosis can contribute to peripheral inflammatory states or trigger downstream metabolic effects that exacerbate the neurodegenerative process in PD. Several preclinical and clinical studies have demonstrated disrupted intestinal permeability, intestinal inflammation, altered gut microbiome, and reduced fecal short-chain fatty acids in PD. In this regard, microbial-directed therapies such as probiotics are emerging as potential therapeutic options. Probiotic supplementation is postulated to confer a variety of health benefits due to the diverse functions of these live microorganisms, including inhibition of pathogen colonization, modulation/"normalization" of the microbiome and/or its function, immunomodulatory effects (e.g. reducing inflammation), and improved host epithelial barrier function. Interestingly, several PD animal model studies have demonstrated the potential neuroprotective effects of probiotics in reducing dopaminergic neuronal degeneration. Notably, two randomized placebo-controlled trials have provided class I evidence for probiotics as a treatment for constipation in PD. However, the effects of probiotics on other PD aspects, such as motor disability and cognitive function, and its long-term efficacy (including effects on PD drug absorption in the gut) have not been investigated adequately. Further targeted animal and human studies are also warranted to understand the mechanisms of actions of probiotics in PD and to tailor probiotic therapy based on individual host profiles to improve patient outcomes in this disabling disorder.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 2–3% of the population above the age of 65 years.[1] With increased life expectancy, there has been a rapid growth in the prevalence of PD, that is, 61% increase in crude prevalence from 1990 to 2017, making it the fastest growing of all the neurological disorders included in the Global Burden of Disease, Injuries, and Risk Factors Study (GBD).[2] This growth has been particularly striking in certain regions of the world, including parts of the Asia-Pacific.[2,3]

PD is a multisystemic disorder characterized by motor symptoms such as slowness of movements (bradykinesia), stiffness (rigidity), tremors, and balance difficulties, as well as a wide spectrum of nonmotor symptoms including gastrointestinal (GI), urogenital, neuropsychiatric, sleep, and pain/sensory disturbances. While symptomatic treatments exist, there is currently no cure for PD, and the physical and mental disabilities caused by this disease pose a major burden to patients, caregivers, and society.

Although the etiology of PD remains elusive, recent advances have suggested that complex interactions between genetic predispositions and exposure to environmental factors, such as toxins and infectious agents, may underlie the selective but widespread loss of neurons in PD.[4] A fundamental role of the GI system, which represents a vulnerable port of entry for pathogens, has been hypothesized in the etiology and progression of this disease.[5,6] In this regard, the intestinal mucosa, which is only millimeters away from enteric nerve terminal endings, represents the largest body surface area exposed to antigens from the external environment and harbors the greatest microbial density in the human body.[7] Studies on the gut–brain axis increasingly suggest an important role of gut microbiota in regulating health and disease via an intense bidirectional interplay of neuronal, immunological, and metabolic signaling.[8] Importantly, these represent potentially modifiable factors in PD, and interventions targeted at this axis could potentially be harnessed to modify the course of this disabling disease.

The "leaky" gut in Parkinson's disease

Aggregation of alpha-synuclein (α-syn) in the nervous system is a neuropathological hallmark of PD. Ever since the discovery of α-syn deposits in the enteric nervous system (ENS) (Auerbach's

and Meissner's plexus) of PD patients in 1988,[9] and subsequent autopsy studies by Braak *et al*. proposing that synucleinopathy may spread within the nervous system via prion-like cell-to-cell transfer,[10] there has been growing evidence that insults acting on the gut could trigger the misfolding and aggregation of α-syn in the enteric nervous system, subsequently propagating into the brain.[5,6,11,12]

A critical aspect to consider in the gut–brain axis theory is the barrier function of the gut epithelium. Indeed, clinical and preclinical studies have demonstrated increased intestinal permeability in PD, and bacterial endotoxin (lipopolysaccharide) exposure has been associated with increased α-syn deposition in the GI tract.[13–15] Disruption of gut barrier function can lead to intestinal inflammation,[16] which was evident in several colonic biopsy and fecal studies on PD.[17–19] Devos *et al*. first showed that PD is associated with intestinal inflammation, with significant elevations in mRNA levels of pro-inflammatory cytokines (tumor necrosis factor-alpha, interferon gamma, interleukin-6, and interleukin-1 beta) and glial markers (Glial fibrillary acidic protein, Sox-10, and S100-beta) in colonic biopsies of patients (n = 19) *versus* controls (n = 14).[17] These changes were akin to those observed in inflammatory bowel disease.[20] Interestingly, several markers of inflammation, including vascular endothelial growth factor receptor 1, interleukin-1α, chemokine (C-X-C motif) ligand 8 (CXCL8), interleukin-1β, and C-reactive protein, were found to be elevated in PD patient-derived stool homogenates (n = 156), compared to non-PD controls (n = 110).[18] In another case–control study (34 PD *vs* 28 controls), Schwiertz *et al*. found elevations of fecal calprotectin (an established fecal marker of intestinal inflammation), as well as alpha-1-antitrypsin and zonulin (markers of intestinal permeability), in PD patients; however, lactoferrin (another marker of intestinal inflammation) was not significantly elevated.[19]

Peripheral inflammatory states such as GI inflammation have been postulated to contribute to neuroinflammation and neurodegeneration in PD.[17] In a series of well-designed experiments comparing α-syn-overexpressing (ASO) mice to germ-free mice, gut microbes were shown to promote neuroinflammatory responses, leading to increased motor disability and α-syn deposition in the brain; remarkably, transplantation of human gut microbiota from PD patients enhanced motor dysfunction in ASO mice.[21] More recently, toll-like receptor-4 (TLR-4) has been highlighted to play a role in intestinal and/or brain inflammation in PD. Loss of TLR4 in a knock-out mouse model significantly mitigated the effects of rotenone (a pesticide widely used in animal models of PD) on intestinal barrier integrity, colonic α-syn deposition, microglial activation, dopaminergic cell loss, and motor function impairment.[22]

Gut microbiome and metabolic changes in Parkinson's disease

With increased accessibility to next-generation sequencing (NGS) technologies, there has been an expansion of studies showing significant, albeit varying, differences in the gut microbiome in PD compared to non-PD controls. These have recently been reviewed by Lubomski *et al*.[16,23,24]

Interestingly, several 16S rRNA gene-sequencing studies have shown significantly reduced abundances of butyrate-producing bacteria in PD fecal samples compared to controls.[25–27] These findings were supported by a quantitative real-time polymerase chain reaction (PCR) study targeting butyrate synthesis genes[28] and a gas chromatography study demonstrating significantly reduced fecal levels of short-chain fatty acids (SCFAs) (butyrate, acetate, and propionate) in PD patients *versus* controls.[29] SCFAs are thought to play a key role in microbiota–gut–brain crosstalk, in part via the regulation of blood–brain barrier integrity, neuronal survival, inflammatory cascades, and endocrine signaling.[30] Butyrate in particular is a major energy substrate for the colonic epithelium and acts to improve gut barrier function.[31] Being a histone deacetylase inhibitor (HDACi), butyrate has been found to exert neuroprotective actions in experimental PD models,[30,32] including the mitigation of PD motor impairment and dopaminergic cell death.[32–36]

Potential roles and mechanistic effects of probiotics

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host.[37] The therapeutic and prophylactic effects of probiotic administration are thought to be mediated through a wide assortment of mechanisms, which have been well described elsewhere,[37–39] and are briefly summarized here.

The host gut microbiota can be impacted by probiotic supplementation through competition (for nutrients and adhesion to the intestinal epithelium), antagonism, and cross-feeding.[37,38] The formation of biofilms (i.e. three-dimensional bacterial communities embedded in self-produced extracellular matrices) by probiotic bacteria promotes colonization and longer permanence in the mucosa of the host and avoids the attachment of pathogenic bacteria.[40] Many probiotic strains are antagonistic toward other microorganisms due to the production of organic acids (e.g. lactic acid by *Lactobacillus* and *Bifidobacterium* species), which lower luminal pH, and also bacteriocins, which inhibit pathogens in the human gut and urinary tract.[41] Interestingly, supplementation with *Lactobacillus* (particularly *Lactobacillus casei*) probiotics during *Helicobacter pylori* eradication therapy has been found to improve eradication efficacy, presumably due to their antagonistic activity against *H. pylori*.[42] Cross-feeding between probiotic bacteria and host microbiota can promote the production of SCFAs such as butyrate in the gut, with benefits as described above.[38]

Probiotics have also been demonstrated to modulate a range of host immune functions, including innate and adaptive (both cell-mediated and humoral) immunity. For example, probiotics can increase phagocytosis and upregulate antibody secretion, translating into improved defenses against pathogens.[38] They can also upregulate various anti-inflammatory factors and downregulate pro-inflammatory cytokines,[43] potentially abating intestinal inflammation. Probiotics have also been shown to improve GI barrier function. For example, *Lactobacillus* and *Bifidobacterium* species can increase the expression of tight junction proteins and upregulate mucus secretion that can help inhibit the adhesion of harmful microorganisms.[38,44] Probiotics strains also improve gut barrier function by downregulating inflammation.

Probiotics are capable of producing a large range of bioactive molecules that affect the host and its microbiota both locally and nonlocally. Microbial-derived neuroactive compounds (or precursors thereof) include oxytocin, gamma-aminobutyric acid (GABA), serotonin, tryptophan, tryptamine, noradrenaline, dopamine, and acetylcholine.[45] The role of probiotics to alleviate symptoms of lactose maldigestion is well known and is mediated by the production of enzymes such as β-galactosidase and bile salt hydrolase, which improve lactose digestion in the host digestive system.[38,39]

Preclinical evidence for probiotic supplementation in Parkinson's disease

Several in vivo animal model studies and cellular model studies have been conducted to evaluate possible neuroprotective effects of probiotics and their potential as a treatment for PD. Among them, murine models were the most widely employed.

Using the genetic MitoPark PD mouse model, Hsieh *et al*. compared the motor functions of mice with and without probiotic supplementation.[46] The control arm was given a sham treatment, while the probiotic treatment arm received a daily mixture of six common probiotic strains (*Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus rhamnosus*, *L. rhamnosus* GG, *Lactobacillus plantarum* LP28, and *Lactococcus lactis* subsp. *Lactis*). The authors detected better motor performance (gait, balance, and coordination) in the treated animals, which were observed as early as week 16 postsupplementation and persisted until the end of the experiment at week 24. In addition, the treatment group exhibited reduced degeneration of nigral dopaminergic neurons, suggesting a neuroprotective effect of the probiotic mixture.

Neuroprotective effects of probiotics were also reported in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- and rotenone toxin-induced PD mouse models.[34] It was found that supplementation with a probiotic cocktail containing *L. rhamnosus* GG, *Bifidobacterium animalis lactis*, and *Lactobacillus acidophilus* promoted the production of butyrate, which subsequently rescued nigral dopaminergic neurons from MPTP- and rotenone-induced neurotoxicity. These effects were consistent with the detection of elevated brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) (these factors promote cell survival and cell proliferation) and the inhibition of monoamine oxidase B (MAO-B) (thus increasing dopamine synthesis and promoting the survival of dopaminergic neurons). In a 6-hydroxydopamine (6-OHDA) mouse model, probiotic (a mixture of nine bacterial strains: *Streptococcus thermophilus*, *B. longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *L. acidophilus*, *L. plantarum*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, and *Lactobacillus. brevis*, formulation SLAB51) conferred neuroprotection with reduced nigral dopaminergic neuronal loss, postulated to be mediated via activation of the peroxisome proliferator-activated receptor gamma (PPAR-γ) by microbial byproducts, subsequently triggering anti-inflammatory and antioxidant activities, as well as upregulation of BDNF and its prosurvival pathways.[47]

Interestingly, probiotics may be genetically modified to augment their desirable effects. For instance, Fang *et al*. gave MPTP-treated PD mice *Lactococcus lactis* subsp. *cremori* that carried a glucagon-like peptide-1 (GLP-1) expression vector.[48] Mice receiving this probiotic exhibited reduced locomotor impairment, increased neurons positive for tyrosine hydroxylase (the enzyme producing dopamine and other catecholamines), and reduced inflammation, in comparison to the control group. In addition, the probiotic also suppressed Enterobacteriaceae (that may act as an intestinal pathogen) and enhanced the number of probiotic *Lactobacillus* and *Akkermansia* species. It is interesting to note that GLP-1 is able to cross the blood–brain barrier to stimulate GLP-1 receptors in the brain; this insulin-signaling pathway is considered to play key functions in neurogenesis, neuronal metabolism, and synaptic plasticity,[49] and GLP-1 agonists are currently the subject of at least eight clinical trials on human PD subjects.[50]

In an α-syn-expressing worm (*Caenorhabditis elegans*) model of PD, feeding *Bacillus subtilis* PXN21 resulted in reduced α-syn accumulation in the host.[51] *B. subtilis*-triggered alterations of host sphingolipid metabolism were found to be the main mechanism involved in the probiotic-driven neuroprotective effect. This finding was in line with increasing evidence in the PD field that an imbalance of lipids, including ceramides and sphingolipids, contribute to PD pathogenesis.[52] In addition, the protective effect of *B. subtilis* was shown to be partly due to biofilm formation in the gut of the worms.[51]

In a cellular model study, Magistrelli *et al*. showed that, by coculturing peripheral blood mononuclear cells (PBMCs) isolated from PD patients with probiotic species (*Lactobacillus salivarius* LS01 DSM 22775, *L. plantarum* LP01 LMG P-21021, *L. acidophilus* LA02 DSM 21717, *L. rhamnosus* LR06 DSM 21981, *Bifidobacterium animalis* subsp. *lactis* BS01 LMG P-21384, and *B. breve* BR03 DSM 16604), the production of pro-inflammatory cytokines was downregulated, while the production of anti-inflammatory cytokines was promoted.[53] Among the tested strains, *L. salivarius* LS01 and *L. acidophilus* displayed the highest activities. Furthermore, the probiotics inhibited the growth of potentially pathogenic bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*.

Clinical evidence for probiotic supplementation in Parkinson's disease

Several studies have demonstrated the benefits of probiotic supplementation in PD patients, particularly as a treatment for constipation. Constipation is a very common symptom in PD, with a reported prevalence of up to 70%.[54] Constipation causes significant distress to many patients and can sometimes lead to serious complications such as intestinal pseudo-obstruction, volvulus, and acute urinary retention.[54] The problem is also often insufficiently responsive to currently available laxative treatments.[55]

In an open-label study of 40 PD patients, supplementation of fermented milk containing *Lactobacillus casei Shirota* (6.5×10^9 colony-forming units [CFU]), together with diet therapy, for 6 weeks was associated with a significant increase in the number of days of bowel opening with normal stool consistency and improvement in constipation-associated bloatedness, sense of incomplete emptying, and abdominal pain.[56]

Two subsequent double-blind placebo-controlled randomized clinical trials (DBPC-RCT) have provided class I evidence for the use of probiotics as a treatment for constipation in

PD.[57,58] In the first RCT, 120 PD patients were randomized to receive either fermented milk containing multiple probiotic strains (consisting of *Streptococcus salivarius* subsp *thermophilus*, *Enterococcus faecium*, *L. rhamnosus GG*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. delbrueckii* subsp *bulgaricus*, *and B. breve* and *animalis subsp lactis*; total 250×10^9 CFU), combined with prebiotic fiber (125 g) ($n = 80$), or a placebo ($n = 40$) for 4 weeks.[57] There was a significant increase in the number of complete bowel movements (defined as a movement after which a feeling of complete evacuation is reported) per week, as measured by stool diary, as well as improvements in bowel frequency, stool consistency, and frequency of laxative usage, in the treatment group.

The second RCT administered multistrain probiotics as a simple once-daily capsule formulation at a lower dose (total 10×10^9 CFU), also for 4 weeks.[58] The formulation consisted of *L. acidophilus*, *Lactobacillus reuteri*, *Lactobacillus gasseri*, and *Lactobacillus rhamnosus*, *B. bifidum* and *B. longum*, *Enterococcus faecalis*, and *E. faecium*. There was a significant increase in the number of spontaneous bowel movements (i.e. not induced by rescue medication) per week, as well as improvements in stool consistency and quality of life associated with constipation in the treatment group ($n = 34$) *versus* placebo ($n = 38$). Probiotic supplementation was not associated with changes in fecal calprotectin. Notably, patients assigned to the treatment group in both trials reported higher satisfaction with the intervention received, compared to placebo-assigned patients.

However, the effects of probiotic supplementation on other disease aspects of PD have not been adequately investigated. In one RCT (Iranian Registry of Clinical Trials 2017082434497N4),[59] supplementation of a multistrain probiotic capsule (combination of *L. acidophilus*, *B. bifidum*, *L. reuteri*, and *Lactobacillus fermentum*; total 10×10^9 CFU) for 12 weeks was reported to be associated with improved disease severity in the treatment ($n = 30$) *versus* placebo groups ($n = 30$). However, the authors reported only collapsed (total) International Parkinson and Movement Disorder Society Unified PD Rating Scale (MDS-UPDRS)[60] scores, without reporting each part (I–IV) separately as has been recommended.[61]

Current gaps and future directions

Modification of the gut microbiome and/or metabolome using dietary,[62] probiotics, and other approaches may provide new therapeutic, and possibly even preventative,[63] options in PD. However, there currently remain many knowledge gaps surrounding the use of probiotics in PD. These offer a rich ground for further studies.

For a start, well-designed and high-resolution investigations on the gut microbiome and especially functional (e.g. metabolomic) alterations in PD need to be conducted, and their findings need to be replicated and further dissected (e.g. in animal and cellular models). In the general probiotic field, insights into probiotic mechanisms of action have mostly been gleaned from in vitro, cell culture, or animal studies,[38] and future research should also incorporate studies investigating their actions in humans (e.g. by ex vivo testing on patient fecal samples or possibly using more invasive methods for in situ sampling of luminal contents and/or mucosal tissues).[39]

The clinical effects of probiotic supplementation on important PD features, including parkinsonian motor disability, motor response complications (e.g. shortened duration of response to medication treatment), and other common nonmotor symptoms such as cognitive impairment, depression, anxiety, and psychosis, are important areas for further research. Longer-term studies examining probiotic efficacy and safety are needed, although these may be quite challenging to perform, particularly in PD (e.g. such studies may require patients to be maintained on stable PD medications over the study duration when, in fact, the typical scenario is often one where disease progression necessitates increases in medication number or dosage). Another major barrier in PD clinical research has been the lack of reliable biomarkers for measuring disease severity and, therefore, the need to rely on clinical rating scales with their inherent limitations.[60,64–67]

A theoretical concern with probiotic supplementation in PD is the potential to induce or exacerbate small intestinal bacterial overgrowth (SIBO), a condition common in PD (presumably due to intestinal dysmotility and possibly also altered host immune function) and one that is associated with worse motor function.[68] Some *Enterococcus* species used in probiotics have also recently been found to inactivate levodopa (the mainstay medication treatment for PD) via their tyrosine decarboxylase enzyme activity[69,70]; the relevance of this finding to PD patients and the safety of probiotics containing such bacterial strains are important issues that await further clarification.

Many questions remain regarding the optimal methods for probiotic delivery, for example, whether (or when) multiple *versus* single strains should be employed and at what combination and dosage.[44] On the one hand, the use of multiple strains has the potential to provide a broader coverage, thus maximizing the chance for efficacy[71] (as different patients may well have differential responses to different species or strains).[72] On the other hand, the use of multiple strains may introduce problems such as antagonism between the administered bacteria,[44,71] which would be counterproductive. Novel and/or recombinant probiotic strains beyond the "typical" *Lactobacillus* and *Bifidobacterium* species are other promising areas for development.[38,39,43] The potential role for prebiotics (defined as substrates that stimulate the growth and/or function of beneficial intestinal microorganisms),[43] combined with probiotics or used alone, needs to be further studied. This is currently a growing field in its own right and was beyond the scope of our review.

Conclusion

In line with the shift in clinical medicine toward personalized therapies,[73–75] much more work is required to be able to rationally tailor probiotic therapies to individual patients, based at least in part on the properties of the baseline host microbiota.[38,39] For example, one could envisage administering SCFA-producing bacteria to PD patients specifically found to be deficient in these compounds (e.g. from fecal or blood assays) or strains with anti-inflammatory activities to patients where intestinal inflammation and/or gut hyperpermeability are thought to play a relevant role in disease pathogenesis.[43] It is hoped that the advent and increasing availability of omics and multiomics approaches (including gut

microbiomics, metabolomics, etc.)[76] will help usher in a new era of optimized precision medicine approaches for our patients.[73]

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