Intestinal dysfunction in Parkinson's disease: Lessons learned from translational studies and experimental models.

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Title page

Intestinal dysfunction in Parkinson's disease: Lessons learned from translational studies and experimental models

Running title: Bowel dysfunctions in Parkinson's disease

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Abstract

Background Symptoms of digestive dysfunction in patients with Parkinson's disease (PD) occur at all stages of the disease, often preceding the onset of central motor symptoms. On the basis of these PD-preceding symptoms it has been proposed that PD could initiate in the gut, and that the presence of alpha-synuclein aggregates, or Lewy Bodies (LBs) in the enteric nervous system might represent one of the earliest signs of the disease. Following this hypothesis, much research has been focused on the digestive tract to unravel the mechanisms underlying the onset and progression of PD, with particular attention to the role of alterations in enteric neurotransmission in the pathophysiology of intestinal motility disturbances. There is also evidence suggesting that the development of central nigrostriatal neurodegeneration is associated with the occurrence of gut inflammation, characterized by increments of tissue pro-inflammatory markers and oxidative stress, which might support conditions of bowel neuromotor abnormalities.

Purpose The present review intends to provide an integrated and critical appraisal of the available knowledge on the alterations of enteric neuromuscular pathways regulating gut motor activity both in humans and pre-clinical models of PD. Moreover, we will discuss the possible involvement of neuro-immune mechanisms in the pathophysiology of aberrant gastro intestinal gut transit and neuromuscular activity in the small and large bowel.

Keywords

Parkinson's disease, colonic motility, small bowel alterations, pre-clinical model, patients

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the general population, with a prevalence of 1% at the age of 55 years, which increases with aging, resulting in an accumulating burden for healthcare. Nigrostriatal dopaminergic loss of neurons, the pathological hallmark of PD, triggers complex functional alterations within the basal ganglia circuitry, which then cause typical motor symptoms (tremor, rigidity, bradykinesia) (1). However, PD is associated also with other peripheral symptoms, that include functional gastrointestinal (GI) abnormalities, consisting mainly of delayed gastric emptying, constipation and anorectal dysfunction (2-4).

GI motility dysfunction often precedes the onset of motor symptoms in patients with PD by many years (5). Furthermore, Lewy bodies (LBs), the typical α-synuclein (α-syn) positive inclusions found in PD brain, have been detected also in neurons of the myenteric plexus and dorsal motor nucleus of the vagus (DMV) of PD patients, suggesting that the underlying pathological process involves also the autonomic nervous system (6). Interestingly, Del Tredici and colleagues postulated that the gut could represent the starting site of PD, and that the presence of LBs in the enteric nervous system (ENS) might be one of the earliest signs of the disease (7). In this context, research efforts are currently focused on understanding the pathophysiological mechanisms underlying gut neuromuscular dysfunctions associated with PD. The present review intends to provide an appraisal of the available knowledge about enteric functional, neurochemical and molecular alterations associated with PD. Special attention has been paid to the pre-clinical PD models would translate to human PD pathophysiology and what role the enteric cell network plays in the pathophysiology of bowel disorders associated with PD.

2. Intestinal enteric abnormalities in PD patients

Infrequent bowel movements and constipation represent the main intestinal disturbances in patients with PD (8). Such disorders may occur both in the early and advanced stages of the disease, worsening the patients' quality of life (9). There is an increasing recognition of the involvement of cells of the enteric neuromuscular compartment in this process (i.e. intrinsic primary afferent neurons, enteric glia, interstitial cells of Cajal), that contribute actively to the regulation of small bowel functions. A number of clinical investigations have attempted to unravel the pathophysiological mechanisms underlying bowel alterations occurring in PD patients. Data on neurochemical, molecular and functional enteric changes in PD patients are discussed in the following sections and summarized in Table 1.

2.1 Small bowel

Little is known about the pathophysiology of small bowel dysmotility associated with PD. Even though the presence of α-syn accumulation has been detected in duodenal and ileal biopsies from PD patients at different stages of the disease, the same biopsies did not reveal any myenteric ganglion loss or alterations of nitric oxide (NO), vasoactive intestinal peptide (VIP), dopamine and catecholamine neuronal density (10-12). Increasingly, the role of enteric luminal microbiota in GI dysfunction associated with brain neurodegenerative and inflammatory diseases (i.e. Alzheimer's disease, PD and multiple sclerosis) is recognized (13, 14). At present, there is some clinical evidence supporting the contribution of enteric bacteria to the alterations of intestinal motility associated with PD. In particular, the occurrence of both small intestine bacterial overgrowth (SIBO) and malabsorption syndrome, characterized by increased bacterial density in the small bowel, with frequent impairment of GI motility, have been observed in PD patients (15, 16). However, the actual involvement of intestinal microbial flora in the pathogenesis of GI abnormalities in PD remains to be elucidated and, most importantly, there is a substantial lack of data on the possible role of the brain-gut-microbiota axis in the pathophysiology of PD. Moreover, it remains unclear whether bacterial overgrowth acts as a possible pathophysiological mechanism, or whether it rather occurs as a mere consequence of the impaired small bowel motility. These are open issues, which represent areas of interest for future investigations.

2.2 Large bowel

Chronic constipation is the most widely recognized functional gut disorder associated with PD. It occurs with a prevalence ranging from 70 to 80% and precedes the onset of movement disorders in 87% of PD patients (5), with negative impact on the patient's quality of life and increased health care costs. PD patients with constipation are characterized by infrequent bowel movements, impairment of propulsive colonic motility and prolonged colonic transit time as well as reduced rectal contractions and abnormalities in motor activity of the anal sphincter (17).

The pathogenic mechanisms underlying the development of colonic constipation associated with PD are presently unclear, as most, if not all, studies fail to demonstrate changes in the neurochemical and/or innervation pattern in intestinal tissue of PD patients. An initial study of human colonic morphological and molecular changes associated with PD was provided by Singaram et al. (18). These authors performed a detailed quantification of submucosal and myenteric dopaminergic neurons by labeling both TH and dopamine. They also assessed the density of VIP neurons, substance P and neuropeptide Y. Their results showed that, in the submucosal plexus, the number of both TH and dopamine containing neurons did not differ from that observed in control tissues, while in the myenteric plexus the number of immunopositive neurons for dopamine were reduced, without significant changes in TH immunostaining. In both plexuses, there were no significant variations seen in the density of

VIP-ergic neurons, SP and neuropeptide Y. The myenteric decrease in the density of dopaminergic neurons was associated with a reduced dopamine content in the muscularis externa, while no differences were detected in the mucosal layer, suggesting an impairment of dopaminergic neurotransmission in the PD human colon (18). Lebouvier et al. (19) confirmed the lack of significant changes in the density of submucosal dopaminergic neurons in colonic biopsies from PD patients. Subsequent investigations by Annerino et al. (11), consistent with the findings reported by Lebouvier et al. (19), did not observe any significant change in the proportion of myenteric nitrergic, VIP-ergic or dopaminergic neurons. Likewise, Corbille et al. (20) did not found abnormalities in the density of dopaminergic neurons as well as the expression levels of dopaminergic and noradrenergic markers in colonic biopsies from PD patients. Taken together, most of current evidence suggests that colonic dopamine, nitrergic and peptidergic pathways are not significantly affected in PD patients. It is interesting to note that neurochemical evidence from non-PD patients with chronic constipation showed distinct profiles of neurotransmitter levels in colonic neuromuscular compartment as compared to PD patients, characterized by a decreased VIP and substance P expression and enhanced nitrergic innervation (21).

When considering the distribution and localization of LBs and Lewy neurites in colonic tissues from PD patients, the presence of α-syn inclusions in colonic neurons has been recently documented at all stages of the disease, suggesting that their accumulation could be viewed as a biomarker of PD (22). However, clear relationships between colonic α-syn accumulation and bowel neuromuscular dysfunctions during PD have not been mechanistically established and should be investigated in the near future.

Over the last years, growing interest has been dedicated to the role played by central and peripheral neuroinflammation in the pathophysiology of neurodegenerative diseases (23). Of note, recent studies, showing a significant increase in pro-inflammatory cytokine levels and enteric glial activation in colonic biopsies from PD patients, suggest that also enteric inflammation could play a key role in the pathogenesis of bowel dysfunctions associated with PD (22, 24, 25). In a recent paper, Keshavarzian et al. (26) analyzed the fecal and mucosal colonic microbiota compositions and showed that PD patients are characterized by a condition of dysbiosis and inflammation. In particular, PD patients displayed decreased abundance of "anti-inflammatory" butyrate-producing bacteria, concomitant with increased levels of "pro-inflammatory" Proteobacteria of the genus *Ralstonia*, that could contribute to promote bowel inflammatory/immune responses during PD (26, 27). In addition, Clairembault et al.(28) have observed morphological alterations of the intestinal epithelial barrier, characterized by reduced levels of occludin in colonic tissues of PD patients (28). Taken together, these observations suggest that alterations of the enteric microbiome, along with changes in intestinal permeability, could contribute to the occurrence of intestinal inflammation observed in PD patients.

Overall, current knowledge points out the presence of aggregated α-syn in enteric neurons, intestinal dysbiosis and the occurrence of inflammatory activity in colonic tissues of PD patients, even though these alterations have not yet been linked to the pathogenesis of colonic motor dysfunctions. It remains also to be conclusively demonstrated whether, and to what extent, impairment of enteric neurotransmission, as well as the immune/inflammatory responses could contribute to colonic dysmotility in PD.

3. Bowel alterations in pre-clinical models of PD

In an attempt to better understand the pathophysiological mechanisms underlying bowel dysmotility occurring in patients with PD, efforts were made to study gut dysfunctions in pre-clinical models of PD. Current models allow two different approaches: 1) ~~peripheral~~ induction of PD-like pathological alterations by systemic administration of neurotoxins; 2) induction of nigrostriatal denervation by central injection of neurotoxins. The main features of PD models employed in studies on the evaluation of GI dysfunctions are summarized in Table 2. In the following sections, data available on functional, neurochemical and molecular intestinal alterations in pre-clinical models of PD are discussed.

3.1 Small bowel

3.1.1 Functional alterations

Current evidence, concerning motor dysfunctions occurring in the small intestinal tract in the presence of PD, is fragmentary and often conflicting (Table 3). In the peripheral model of dopaminergic degeneration induced by 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, which blocks mitochondrial complex I activity into dopaminergic neurons of the substantia nigra pars compacta) in mice, Anderson et al. (29) did not detect any significant alteration in small intestinal transit. In PD induced by central injection of toxins, only one study, showing alterations of intestinal transit, is available. In particular, a delayed transit in the distal region of small bowel has been observed by means of geometric center analysis in rats injected with 6-OHDA, suggesting that central dopaminergic denervation is associated with impaired small bowel motility (30). However, given the paucity of data, extensive investigations are needed to better understand the impact of central dopaminergic neurodegeneration on the onset of intestinal dysmotility.

3.1.2 Neurochemical and molecular alterations

3.1.2.1 Inhibitory pathways

Most of the available knowledge about the alterations of enteric neurotransmission in small bowel during PD concerns changes of the dopaminergic network in both peripheral and central models of dopaminergic neurodegeneration (Table 3, Figure 1). In particular, mice treated with MPTP displayed a significant decrease in the number of dopamine transporter (DAT) and tyrosine hydroxylase (TH)-positive neurons in both myenteric and submucosal plexus of the duodenum and ileum (29). Likewise, molecular analysis showed a reduced protein expression of TH and DAT in the duodenum of MPTP-treated mice (31). These findings are in keeping with a subsequent report by Natale et al. (32), who observed a significant reduction of dopamine content in the duodenum of MPTP-treated mice, suggesting that this peripheral model is characterized by an impairment of small bowel dopaminergic neurotransmission.

When considering PD elicited by central toxins, only one study examined the alterations of dopaminergic markers in intestinal tissues from rats with 6-hydroxydopamine (6-OHDA)-induced PD. This work showed a significant increase in TH and DAT expression (31), in contrast to data obtained from the MPTP model, where a decrease in enteric dopaminergic markers was evident. These differential rearrangements of the enteric dopaminergic network could depend on intrinsic differences of PD models in different animal species, which might also reflect different pathophysiological stages of the disease.

There is scarce evidence on the neurochemical modifications of enteric nitrergic and VIP-ergic inhibitory nerve pathways in the presence of PD. Of note, no changes in the number of both enteric nitrergic and VIP-ergic neurons were detected upon central denervation induced by peripheral toxins (29, 32-34). In the setting of 6-OHDA-induced nigrostriatal neuro-degeneration, only two studies have examined the possible alterations of nitrergic and VIP-ergic pathways in the small intestine. Colucci et al. (35) observed a decrease in the density of neuronal nitric oxide synthase (nNOS) positive neurons along with an increased percentage of VIP-immunoreactive neurons in the myenteric plexus of distal ileum, while Toti and Travagli (36) showed an increase in the density of nNOS neurons in the duodenum. These results suggest that changes in the expression of nitrergic neuronal markers could vary depending on the intestinal region considered. However, additional investigations are needed to better understand the involvement of nitrergic pathways in intestinal dysfunctions associated with PD.

3.1.2.2 Excitatory pathways

Information on the alterations of small bowel cholinergic excitatory neurotransmission in experimental models of PD are quite limited and heterogeneous (Table 3). For instance, no changes in the number of myenteric cholinergic neurons were observed in the duodenum and ileum of mice treated with MPTP (29, 32). In the 6-OHDA model of central dopaminergic degeneration, Colucci et al. (35) did not detect any variation in the number of cholinergic enteric neurons, while Toti and Travagli (36) reported a decrease of this neuronal population, likely as a result of the different bowel regions and timing. Indeed, Toti and Travagli (36) performed their experiments on the duodenum 5 weeks after the induction of central neurodegeneration, while Colucci et al. (35) performed their assays in the distal ileum from rats sacrificed 4 weeks after 6-OHDA injection. Thus, it appears that in this model the alterations of enteric cholinergic neurons are more likely to occur in the proximal sections of small bowel.

3.1.3 Discussion

Taken together, current data suggest that in the presence of central dopaminergic neurodegeneration the small intestinal transit is impaired, likely as a consequence of alterations in the chemical coding of enteric inhibitory neurons. However, there is a lack of consistent data concerning the involvement of cholinergic, nitrergic and VIP-ergic pathways in small bowel dysfunctions during PD. Likewise, it remains still unclear whether enteric dopamine plays a role in intestinal alterations associated with central dopaminergic neurodegeneration, since a characterization on the physiological role of dopamine in the control of small bowel motility is still lacking. Furthermore, there is no detailed information about the pathophysiological mechanisms underlying the development of intestinal dysmotility in PD. Based on this picture, the effects of central neurodegeneration on small bowel motor abnormalities remain a field largely opened to future investigations.

3.3 Large bowel

3.3.1 Functional alterations

A number of studies have evaluated the abnormalities of colonic motility associated with central dopaminergic neurodegeneration (Table 3). Two reports described a delay of *in vivo* colonic transit and constipation in rotenone- and MPTP-induced neurodegeneration in mice (32, 33). Functional *in vitro* experiments demonstrated an increase in contractile activity and an impaired relaxation in the proximal colon from MPTP-treated mice, suggesting an alteration in the inhibitory control of colonic motility (29, 33). Likewise, in the central 6-OHDA model, a significant decrease in stool frequency and delay in colonic transit were detected (37, 38). In addition, an e*x-vivo* analysis of peristalsis displayed an altered pattern of colonic longitudinal muscle contraction and a reduced peak pressure, suggesting that central dopaminergic neurodegeneration is associated with an impairment of colonic motility resulting in a reduced efficiency of the peristaltic reflex (35). These findings have been corroborated by recent *in vitro* functional studies, showing an impairment of colonic spontaneous contraction and a decrease in electrically evoked cholinergic contractions of both longitudinal and circular colonic smooth muscle from 6-OHDA rats (38, 39). However, in the same model, Pellegrini et al. (40) described also an enhanced *in vitro* longitudinal colonic tachykininergic contractile activity. This apparent discrepancy with a reduced peristaltic activity can be explained by the fact that the patterns of *in vivo* colonic transit do not necessarily reflect the contractile responses elicited by specific stimulation of the excitatory tachykininergic pathway, but rather can be viewed as a result of an integrated combination of smooth muscle contractions regulated by both excitatory and inhibitory neurotransmitter pathways.

3.2 Neurochemical and molecular alterations

3.2.1 Inhibitory pathways

Changes in both inhibitory and excitatory enteric pathways regulating colonic motility in experimental PD have been evaluated by morphological investigations (Table 3). In the PD model elicited by rotenone (an isoflavone insecticide), no changes in nitrergic, VIP-ergic and dopaminergic enteric neuron number or innervation quality were detected (33). By contrast, in the setting of PD induced by intranigral injection of 6-OHDA, alterations of inhibitory enteric pathways were observed. In particular, increments of VIP-ergic and dopaminergic neurons in concomitance with a decrease in nitrergic neurons were detected in the proximal colon (35, 37, 41). Colucci et al. (35) showed that dopamine D_2 receptors were mostly expressed in enteric cholinergic and dopaminergic neurons, and that their immunoreactivity was markedly reduced in myenteric neurons of both proximal and distal colon. In addition, in two previous studies (39, 42) an increase in dopamine content was observed in the colonic muscularis externa of 6-OHDA rats, which could contribute to colonic dysmotility in PD. Zhang et al. (39) observed also an increase in adrenergic β_3 receptor expression and a decreased expression of serotoninergic 5-HT_4 receptors, suggesting that neurochemical and molecular changes affecting the enteric pathways might contribute to colonic dysmotility and constipation displayed by 6-OHDA rats.

Additional support to the concept that alterations of inhibitory dopaminergic and nitrergic pathways can contribute to the occurrence of colonic dysfunctions associated with experimental PD has been provided by molecular studies. When considering the dopaminergic network, a study by Tian et al. (31) showed that, in the MPTP mouse model, the colonic TH protein levels were decreased, while in the 6-OHDA rat model an increase in the protein levels of TH and DAT was found. The latter observations are consistent with those reported by Zhu et al. (37), who showed an increase in TH protein levels in the proximal colon of 6-OHDA rats, suggesting that differential changes in the colonic dopaminergic system can occur depending on the different experimental models of PD. Interestingly, central dopaminergic denervation is associated also with an impairment of colonic nitrergic pathways, as documented by a significant decrease in nNOS protein levels in the proximal colon of PD rats (37).

3.2.1 Excitatory pathways

At present, there is inconsistent data on putative changes occurring in the colonic excitatory cholinergic system during PD (Table 3, Figure 2). Only one study has shown alterations of cholinergic enteric neurons in the PD model induced by peripheral toxins. In particular, in the rotenone model, ChAT immunoreactivity was significantly decreased in the myenteric plexus after 1 and 6 weeks from toxin infusion (43). When considering PD induced by 6-OHDA, the evidence showing changes in the cholinergic excitatory pathway are fragmentary and conflicting. Two studies did not show alterations in the density of ChAT-positive neurons, as well as in ChAT protein and mRNA expression levels in the proximal colon from 6-OHDA rats (35, 37). Conversely, in a recent paper by Fornai et al. (38) a decrease in ChAT immune positivity in the distal colon of 6-OHDA rats was detected. Furthermore, a significant decrease in acetylcholine release from colonic preparations was observed, suggesting an impairment of colonic cholinergic neurotransmission in the presence of central dopaminergic neurodegeneration. In the same study, additional molecular analysis showed an increased expression of muscarinic M_2 and M_3 receptors in the colonic neuromuscular layer and isolated colonic smooth muscle cells from 6-OHDA rats, thus indicating an up-regulation of muscular muscarinic receptors as a compensatory response to the impairment of cholinergic neurotransmission (38). Consistently with these findings, *in vitro* functional experiments displayed an impaired colonic motility in PD rats (38). More recently, Pellegrini et al. (40) observed significant changes in the tachykininergic pathway of the colonic neuromuscular layer from 6-OHDA rats. In particular, nigrostriatal degeneration was followed by an increase in endogenous substance P expression in myenteric ganglionic neurons, along with an enhancement of NK_1 receptors in longitudinal muscle cells, thus indicating that the enteric excitatory tachykininergic pathway can be affected also by central dopaminergic neurodegeneration.

3.1.3 Discussion

An overall appraisal of current data suggest that nigrostriatal neurodegeneration is followed by an impairment of distal colonic motility, leading to a reduced efficiency of peristaltic reflex. Such alterations seem to result from a rearrangement in the chemical coding of enteric inhibitory and excitatory neurons, since a loss of neurons in the colonic myenteric plexus has not been detected (38). In particular, the impairment of cholinergic neurotransmission appears to playa significant role in the onset of colonic dysmotility during PD. However, the roles played by enteric nitrergic and dopaminergic neurotransmission in colonic dysmotility associated with PD remains scarcely understood and deserve further investigations.

4. Overall conclusions and future directions

Human studies and evidence from pre-clinical models suggest that PD is associated with significant functional, neurochemical and molecular alterations throughout the intestinal tract.

Studies in animal models support the view that intestinal motor abnormalities are associated with changes in the neurochemical coding of myenteric neurons, both in the small and large bowel. Most of functional pre-clinical findings are consistent with bowel dysfunctions observed in patients with PD, even though current morphological and molecular data on both inhibitory and excitatory pathways regulating enteric motility are fragmentary and often conflicting. In addition, a detailed characterization of abnormalities of bowel motor pathways occurring in PD is lacking. It remains also unclear whether the occurrence of rearrangements in the enteric neuronal network and intestinal dysfunctions are direct consequences of the central neurodegeneration or could represent the starting point of the disease. In this respect, a crucial role seems to be played by the neuro-immune brain-gut axis, particularly for DMV, that provides parasympathetic innervation to the majority of GI tract and is regarded as one of the two CNS sites displaying the earliest pathological involvement in PD (44). Indeed, the functional and neurochemical changes affecting the ENS, following central dopaminergic denervation, have been shown to directly result from alterations occurring in the DMV, which is modulated by brainstem dopaminergic circuitries and represents a prominent target of neurodegenerative PD-related processes (45, 46). In support of this view, a reduced expression of ChAT in the DMV of rats with 6-OHDA-induced PD and a reduced digestive motor activity have been reported. In addition, when animals were subjected to vagotomy, the digestive dysmotility improved, thus suggesting that gut dysfunctions in PD could depend on the impairment of vagal brain-gut axis (47).

The vagus nerve is referred also as the “cholinergic anti-inflammatory pathway”, since it seems to exert tonic anti-inflammatory actions, while vagotomy confers an increased susceptibility to the development of inflammatory motility disturbances (48). In the setting of PD, two recent pioneering studies, performed both in colonic biopsies from PD patients and colonic tissues from 6-OHDA-induced PD in rats, have shown an increase in pro-inflammatory cytokine levels and enteric glial activation (24, 40). In addition, the pre-clinical study demonstrated also an increase in colonic tissue oxidation, an increment of neutrophil and mast cell density, and a polarization of peritoneal macrophages towards a pro-inflammatory phenotype (40). These results suggest the occurrence of an inflammatory condition in the gut during PD, which could result from an impairment in the vagal brain-gut axis, and might contribute to generate or worsen enteric dysfunctions related to central neurodegeneration (24). Since the extrinsic vagal impairment is expected to affect the overall bowel functions, the development of tissue inflammatory response should affect the whole intestinal tract. On the other hand, as in the CNS, changes in the ENS of PD patients or animal models might be restricted to functionally specific and limited bowel regions or ganglia, and this outcome might increase the likelihood of under- or over-estimating the actual gut alterations. However, current state of the art does not allow to accept or rule out any of the above hypotheses, and therefore this issue remains open to future investigations aimed at evaluating the molecular, morphological and functional changes occurring in different intestinal regions, at different time points, in different experimental models and PD patients.

In conclusion, current knowledge encourages research efforts dedicated to clarify the role played by neuro-immune brain-gut axis in the pathogenesis of gut motor dysfunctions in PD. In particular, it appears of primary relevance to investigate whether gut neuro-inflammation is a consequence of the impairment of vagal cholinergic anti-inflammatory pathway, and whether such a condition could be responsible for the alterations of gut motor pathways occurring in the presence of central dopaminergic neurodegeneration.

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AUTHOR CONTRIBUTIONS

CP, MF and LA wrote the first draft of the manuscript. CP prepared the figures. EB, RC, VB, NB, WdJ and CB revised the manuscript

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Table 1. Functional, neurochemical and molecular alterations associated with bowel disorders in PD patients

Region	***Disorder***	***Observations***	***Ref.***
Small bowel	*Dysmotility*	✓ α-syn accumulation in mucosal submucosal nerve fibres and myenteric ganglia of duodenum and ileum ✓ No alterations of nitrergic, VIPergic, dopaminergic and adrenergic neuron density in myenteric neurons ✓ Small intestine bacteria overgrowth	[10] [11] [12] [16]
Large bowel	*Constipation* *Anorectal dysfunction*	✓ Reduction of dopamine levels in submucosal and myenteric plexus ✓ No alterations of neuronal density in submucosal and myenteric plexus ✓ No alterations of nitrergic, VIPergic, dopaminergic and adrenergic neuron density in myenteric neurons ✓ Aggregated α-syn in cholinergic and substance P-containing neurons in submucosal and mucosal biopsies ✓ Increase in cytokine levels (TNF, IL-1β, IL-6, IFN-γ) and enteric glial activation (GFAP and Sox-10)	[11] [18] [19] [24] [25] [49] [50]

Abbreviations: α-syn: α-synuclein; GFAP: glial fibrillary acidic protein; IL-1β: interleukin 1 beta; IL-6: interleukin-6; IFN-γ: interferon gamma; Sox-10: sex determining region Y (SRY) box containing gene 10; TNF: tumor necrosis factor; VIP: vasoactive intestinal peptide.

Table 2. Main features of experimental models employed in the evaluation of bowel alterations associated with central dopaminergic neurodegeneration

Experimental models	***Functional, neurochemical and molecular characteristics***		***Ref.***
Neurodegeneration induced by peripheral toxin administration	MPTP	✓ Blockade of mitochondrial complex I in SNpc ✓ Loss of dopaminergic neurons in the SNpc ✓ Intraperitoneal administration not associated with formation of LB-like cytoplasmic inclusions ✓ Administration via osmotic minipumps followed by formation of LB-like cytoplasmic inclusions containing ubiquitin and α-syn	[51]
	Rotenone	✓ Different routes of administration (oral, intraperitoneal, intravenous and systemic by osmotic pump) ✓ Degeneration of nigrostriatal dopaminergic neurons ✓ Blockade of mitochondrial electron transport chain ✓ Increase in oxidative stress ✓ Inhibition of proteasome activity ✓ LB-like cytoplasmic inclusions containing ubiquitin and α-syn	[51] [52] [53]
Neurodegeneration induced by intranigral toxin administration	6-OHDA	✓ Bilateral or unilateral intranigral administration ✓ Massive anterograde degeneration of the nigrostriatal pathway (nigral cell loss and striatal dopamine depletion) ✓ Increase in hydrogen peroxide formation ✓ Inhibition of mitochondrial complex I in SNpc	[54]

Abbreviations: α-syn: α-synuclein; LB: Lewybodies; MPTP: 1-methyl-4-phenil-1,2,3,6,tetrahydropyridine; 6-OHDA: 6-hydroxydopamine; SNpc: substantia nigra pars compacta

Table 3. Summary of functional, neurochemical and molecular evidence in the small and large bowel associated with central dopaminergic neurodegeneration

Experimental models (Species)	***Functional evidence***	***Neurochemical evidence***	***Molecular evidence***	***Ref.***
Small bowel				
MPTP (mouse)	Reduced myoelectric activity Prolonged irregular contractions No alterations of overall transit	↓ TH ↓ DAT No alterations of VIP No alterations of NET	↓TH protein expression ↓DAT protein expression ↓DA concentration	[29] [31] [32]
Unilateral intranigral injection of 6-OHDA (rat)	Reduced transit	↓nNOS (distal ileum) ↓nNOS (duodenum) ↑ VIP No alterations of ChAT (distal ileum) ↓ChAT (duodenum) No alterations of HuC/D expression	↑TH protein expression ↑DAT protein expression	[31] [35] [36]
Large bowel				
MPTP (mouse)	Delay of transit Impaired inhibitory control of motility	n.a	↓TH protein expression	[29] [31] [32]
Rotenone (mouse)	Delay of transit	No alterations of VIP No alterations of NOS No alterations of TH	n.a.	[33]
Unilateral intranigral injection of 6-OHDA (rat)	Decrease in stool frequency Delayed colonic transit Reduced efficiency of peristaltic reflex Alterations of longitudinal muscle contraction Decrease in electrically evoked cholinergic contractions	↑ VIP ↑ TH ↓ nNOS ↓ D_2 dopaminergic receptors No alteration of ChAT (proximal colon) ↓ ChAT (distal colon)	↑ TH protein expression ↑ DAT protein expression ↓ nNOS protein expression ↓ nNOS mRNA expression ↓ ChATimmunopositivity ↓ Acetylcholine release from enteric neurons ↑ M2 and M3 muscarinic receptor expression	[29] [35] [37] [38] [41]

Abbreviations: ChAT: choline acetyltransferase; DAT: dopamine transporter; DA: dopamine; NET: noradrenaline transporter; nNOS: neuronal nitric oxide synthase; TH: tyrosine hydroxylase; VIP: vasoactive intestinal peptide; n.a: data not available.

Figure legends

Figure 1. Schematic illustration of small bowel abnormalities in experimental models of Parkinson's disease (PD). Left panel: central dopaminergic denervation, induced by intranigral toxins injection, is associated with an increase in choline acetyl transferase (ChAT) and nitric oxide (NO) expression in myenteric neurons of duodenum. In the distal ileum, the expression of tyrosine hydroxylase (TH), dopamine transporter (DAT) and vasoactive intestinal peptide (VIP) is increased in both myenteric and submucosal plexus, while the expression of neuronal nitric oxide synthase (nNOS) is decreased in myenteric neurons. The overall small intestinal transit is reduced. Right panel: central dopaminergic denervation induced by peripheral toxins injection is associated with a decrease in DAT and TH expression in both myenteric and submucosal plexus of duodenum and ileum. No changes in small intestinal transit have been reported.

Figure 2. Representative picture showing changes in colonic cholinergic neurotransmission in the presence of central dopaminergic denervation elicited by 6-OHDA. The expression of choline acetyltransferase (ChAT) in myenteric plexus is decreased, as well as acetylcholine release from colonic myenteric nerves, in comparison with normal animals. The expression of muscarinic M_2 and M_3 receptors in the colonic smooth muscle layer is enhanced. The colonic motor activity is impaired.

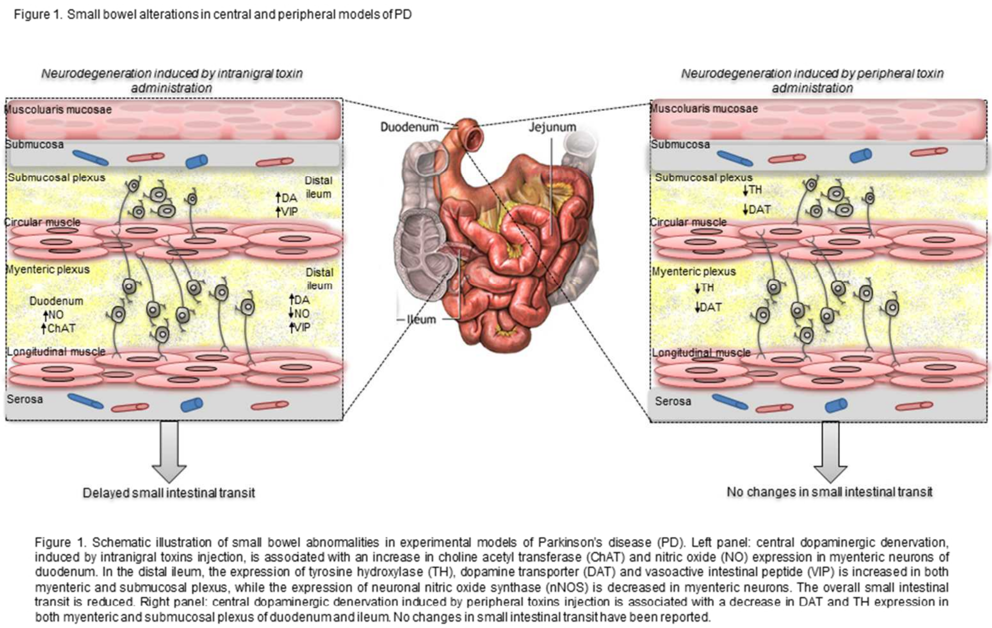

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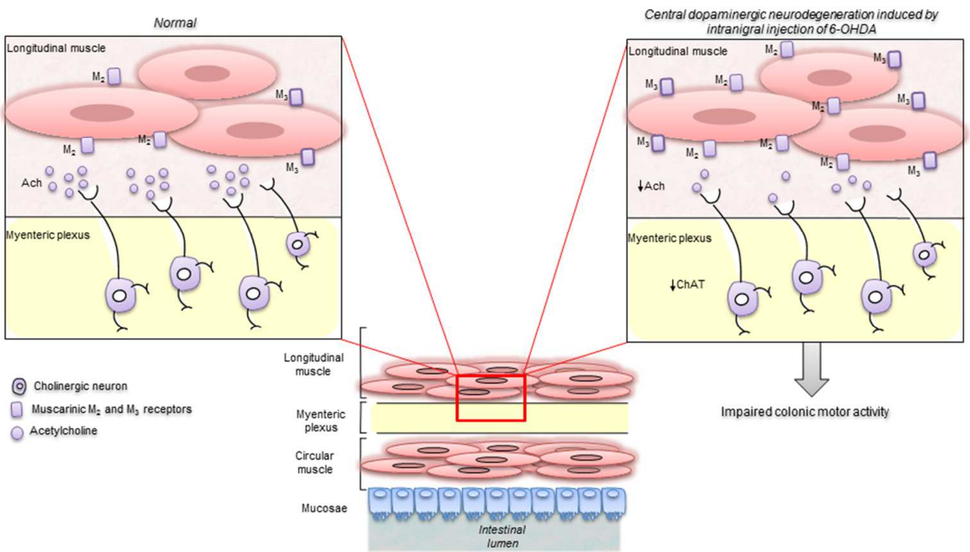
Figure 2. Alterations of colonic cholinergic neurotransmission in the presence of central dopaminergic denervation induced by intranigral injection of 6-OHDA



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Key points

- Infrequent bowel movements and overt constipation represent the main intestinal disturbances in patients with PD.
- PD patients are characterized by aggregated α-syn in enteric neurons, intestinal dysbiosis and the occurrence of inflammatory activity in colonic tissues.
- The implementation of preclinical PD models is allowing a characterization of the role played by the enteric cell network in the pathophysiology of bowel disorders associated with PD.
- Studies in animal models support the view that intestinal motor abnormalities are associated with changes in the neurochemical coding of myenteric neurons, both in the small and large bowel.