Review

Parkinson’s disease from the gut

Rodger A. Liddle *

Department of Medicine, Duke University Medical Center and Department of Veterans Affairs Health Care System, Durham, NC 27710, United States

ABSTRACT

Parkinson’s disease (PD) is a debilitating neurodegenerative condition associated with tremor, rigidity, dementia, and gastrointestinal symptoms such as constipation, nausea and vomiting. The pathological hallmarks of PD are Lewy bodies and neurites in the brain and peripheral nerves. The major constituent of Lewy bodies is the neuronal protein α-synuclein. Misfolding of α-synuclein confers prion-like properties enabling its spread from cell to cell. Misfolded α-synuclein also serves as a template and induces misfolding of endogenous α-synuclein in recipient cells leading to the formation of oligomers that progress to fibrils and eventually Lewy bodies. Accumulating evidence suggests that PD may arise in the gut. Clinically, gastrointestinal symptoms often appear in patients before other neurological signs and aggregates of α-synuclein have been found in enteric nerves of PD patients. Importantly, patients undergoing vagotomy have a reduced risk of developing PD. Experimentally, abnormal forms of α-synuclein appear in enteric nerves before they appear in the brain and injection of abnormal α-synuclein into the wall of the intestine spreads to the vagus nerve. Ingested toxins and alterations in gut microbiota can induce α-synuclein aggregation and PD, however, it is not known how PD starts. Recently, it has been shown that sensory cells of the gut known as enteroendocrine cells (EECs) contain α-synuclein and synapse with enteric nerves, thus providing a connection from the gut to the brain. It is possible that abnormal α-synuclein first develops in EECs and spreads to the nervous system.

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1. Introduction

Patients with Parkinson’s disease (PD) suffer from well-recognized motor disturbances including slow movements (bradykinesia), resting tremor, rigidity, and postural instability. Also common are non-motor symptoms such as loss of smell, sleep disorders and gastrointestinal symptoms, particularly constipation and gatropareisis (Abbott et al., 2001; Cersosimo et al., 2013; Haehner et al., 2009; Irazo et al., 2006; Jost, 2010). PD is a progressive neurodegenerative disease and is often associated with depression. Interestingly, the non-motor symptoms may precede the manifestation of classic motor disturbances by over a decade (Klingelhofer and Reichmann, 2015).

The key pathological features of PD are selective degeneration of dopaminergic neurons of the substantia nigra pars compacta and distinctive α-synuclein-containing cytoplasmic inclusions known as Lewy bodies (Angot and Brundin, 2009; Bisaglia et al., 2009; Soto, 2012). Loss of dopaminergic neurons is responsible for the distinctive movement disorder and vagal nerve dysfunction.

Alpha-synuclein is the major protein component of Lewy bodies within the soma, axons or dendrites of neurons and is believed to play a pathological role in the progression of PD. Alpha-synuclein is a 140 amino acid protein that has a propensity to misfold and aggregate (Angot et al., 2012). It is believed that misfolded α-synuclein has the ability to spread from cell-to-cell in a prion-like manner (Desplats et al., 2009; Goedert, 2015; Hansen et al., 2011; Iuk et al., 2012; Olano et al., 2009). When taken up by another neuron, misfolded α-synuclein can serve as a template for the misfolding of other endogenous α-synuclein molecules (Angot and Brundin, 2009; Goedert, 2015; Steiner et al., 2011) in the recipient cell (Fig. 1). Accumulation of α-synuclein aggregates leads to the formation of oligomers, fibrils, and ultimately Lewy bodies. Although originally described in the brain, aggregated α-synuclein is also found in the peripheral nerves including the enteric nervous system.

Although the ability of misfolded α-synuclein to spread and propagate in a prion-like fashion is well established, it is important to note that it is likely that other regional or cell-autonomous factors are also involved that would account for the regional distribution of PD pathology (Surmeier et al., 2017b). For example, neurons are extremely heterogeneous and those affected in PD are notable for having particularly long and highly branched axons. In addition,
it appears that dopaminergic neurons of the substantia nigra exhibit particularly high mitochondrial stress and cytosolic calcium levels which promote α-synuclein aggregation (Rom-’H-chen-Gauthier et al., 2014; Surmeier et al., 2017a). Therefore, the spread of pathogenic α-synuclein together with endogenous factors that render neurons susceptible to damage appear to be responsible for the neurotoxicity seen in Parkinson’s disease.

Evidence that Parkinson’s disease may originate in the gut has been the topic of several excellent recent reviews (Borghammer, 2017; Lionnet et al., 2017; Surmeier et al., 2017b) and is briefly summarized below. The current article proposes a possible explanation for how PD may originate in the gut in light of the recent unveiling of an enteroendocrine cell – neural circuit and the discovery of α-synuclein in enteroendocrine cells (Bohorquez et al., 2015; Chandra et al., 2017).

2. Evidence for PD in the gut

Up to 30% of patients with PD suffer from gastrointestinal symptoms (Pfeiffer, 2003), the most common of which are nausea, vomiting, and constipation (Martinez-Martin, 2011). Although, motility disturbances of the stomach and colon are frequent, any portion of the gastrointestinal tract may be affected (Lang, 2011; Stern and Siderowf, 2010). Interestingly, the onset of constipation usually precedes the motor symptoms of PD (Abbott et al., 2001; Savica et al., 2009) and worsens with disease progression (Edwards et al., 1991). Prolonged colonic transit time has been documented in the disease and are consistent with constipation symptoms (Jost, 2010). Moreover, the development of constipation is independent of age or physical activity (Abbott et al., 2001).

Pathological studies have revealed a chronological and anatomical progression of PD (Braak et al., 1996; Gaspar and Gray, 1984). Identification of neuropathological changes in olfactory neurons and the vagus nerve suggested that PD may spread from peripheral sites before affecting the brain (Braak et al, 2003a,b; Braak and Del Tredici, 2009). Notably, α-synuclein has been shown to be transported in both anterograde and retrograde directions in the vagus nerve (Ulusoy et al., 2013; Ulusoy et al., 2017). Braak and colleagues hypothesized that aberrant α-synuclein accumulation begins in the gut and progresses via the vagus nerve to the brain in a prion-like manner following ingestion of a neurotropic pathogen leading to PD (Del Tredici and Braak, 2008; Hawkes et al., 2009; Reichmann, 2011). Currently, the pathogenesis of PD remains incompletely understood, nevertheless there is considerable evidence that the enteric nervous system is a site of early involvement (Borghammer, 2017; Braak et al., 2003b; Del Tredici et al., 2002). For example, α-synuclein inclusions have been found in submucosal and myenteric neurons (Braak et al., 2003a, 2006; Braak and Del Tredici, 2009) and α-synuclein aggregates seem to appear in enteric nerves before they are found in the brain (Braak et al., 2003b; Braak and Del Tredici, 2009; Corbille et al., 2016a; Hawkes et al., 2010; Poulet et al., 2012).

Clinical epidemiological evidence supports the concept that PD arises in the gut and spreads to the brain via the vagus nerve. In two studies spanning up to 30 years, it was observed that patients who had a trunical vagotomy (in which the abdominal vagus nerve was transected) but not a highly selective vagotomy (in which only the upper portion of the gastric vagus nerve was transected) had a lower risk of developing PD than the normal population (Liu et al., 2017; Svensson et al., 2015). These studies indicate not only that vagotomy reduces the risk of PD but suggest that the vagus nerve is involved in the transmission of PD and support the concept that PD arises in the gut.

PD is a multifactorial disease with a strong environmental component. Less than 10% of PD is inherited. Environmental exposure to herbicides and pesticides have been associated with an increased risk of PD and high levels of α-synuclein in the brain (Chen et al., 2013; Goldman, 2014; Tanner et al., 2011). The mechanism of toxicity appears to involve inhibition of mitochondrial function or induction of oxidative stress (Tanner et al., 2011). Experimentally, in mice, intragastric administration of the pesticide rotenone, which inhibits mitochondrial complex I activity, induced parkinsonian-like neuropathological changes that were first seen in the enteric nervous system and only later in the substantia nigra pars compacta (Pan-Montojo et al., 2010). Systemic levels of drug were undetectable implying that rotenone induced parkinsonian changes through a local site of action (i.e., the gastrointestinal tract).

In addition to acting locally on the enteric nervous system to mimic PD-like pathology (Pan-Montojo et al., 2010), rotenone’s effect on PD-like disease progression was prevented by hemivagotomy or resection of autonomic nerves (Pan-Montojo et al., 2012). Moreover, rotenone caused the release of α-synuclein from enteric neurons where it was taken up by presynaptic neurites and transported in a retrograde fashion to the dorsal motor nucleus of the vagus. These findings indicated that pesticides like rotenone can promote Parkinson’s disease progression. This concept was further supported by observing the active transport of α-synuclein from the intestine to the brainstem following injection of monomeric or oligomeric α-synuclein into the intestinal wall in rats (Holmqvist et al., 2014) and are consistent with the early appearance of Lewy bodies in neurons projecting from the vagus nerve to PD (Braak et al., 2003b). Moreover, transport of α-synuclein in a caudo-rostral direction via the vagus nerve has also been demonstrated following direct injection of adeno-associated viral vectors overexpressing human α-synuclein into the vagus nerve (Ulusoy et al., 2013).
In order for α-synuclein to propagate, nerves bearing α-synuclein must reside in close proximity to recipient nerves also containing α-synuclein. This association exists in the enteric nervous system where submucosal, myenteric neurons and preganglionic parasympathetic neurons all express α-synuclein (Chandra et al., 2017; Phillips et al., 2008). Thus, there is a potential neural pathway for α-synuclein transmission from the intestine to the brain.

In the intestine, α-synuclein exists in a monomeric form (Corbille et al., 2016b) and may be released from neurons into the paracellular space as either free protein or in the form of exosomes (Danzer et al., 2012). It may then be taken up by neighboring neurons via endocytosis (Angot et al., 2012; Desplats et al., 2009). In humans, however, PD lesions were observed in pre-ganglionic sympathetic neurons suggesting that α-synuclein may be transported trans-synaptically from neuron to neuron. In experimental models of PD, only neurons with intact synaptic connections transported α-synuclein in a retrograde fashion from enteric nerves to the vagus nerve (Pan-Montejo et al., 2010).

Despite the evidence incriminating the enteric nervous system in the pathogenesis of PD, how orally administered agents induce changes in enteric nerves is unknown since enteric nerves extend to the submucosa of the intestine but do not penetrate into the intestinal lumen. Thus, nerves are not directly exposed to contents within the gut lumen. Although PD may arise in the gut, how ingested environmental toxins produce abnormal α-synuclein within enteric neurons is unknown (Bétarbet et al., 2000; Braak et al., 2003b; Braak and Del Tredici, 2009; Hawkes et al., 2010; Holmqvist et al., 2014; Lebourvier et al., 2009; Natale et al., 2008).

3. EEC – neural circuit

Enteroendocrine cells (EECs) are electrically excitable, chemosensorial cells that reside within the mucosa of the gastrointestinal tract. They are best known for the hormones they produce and their anatomical orientation - with an apical surface that is exposed to the lumen of the gut and a basal portion that contains abundant secretory granules. This orientation allows EECs to respond to signals such as ingested nutrients or bacteria in the lumen of the gut. Such stimuli release hormones from the basal surface. Recently, and somewhat surprisingly, EECs were discovered to possess many neuron-like features including neurotrophic receptors, pre- and post-synaptic proteins, and neurofilament-containing axonal-like processes called neuropods (Bohórquez et al., 2014). They also possess the enzymatic machinery for dopamine synthesis. Expression of synaptic proteins raised the possibility that EECs come into contact with nerves. This possibility was subsequently established using a retrograde rabies virus tracing technique demonstrating that EECs synapse with submucosal enteric nerves (Bohórquez et al., 2015). Thus, a neural circuit connects the gut lumen with the nervous system.

This newly recognized EEC-neural circuit raises a number of interesting possibilities. First, EECs can receive stimuli from the gut lumen, respond to those signals, and send messages to enteric nerves and eventually the brain via release of hormones or neurotransmitters. Second, as exemplified by their infection with rabies virus, EECs may be a portal for the entry of pathogens into the nervous system. And third, by virtue of their neuron-like properties, EECs may be subject to neuronal-type abnormalities. This latter possibility was raised with our recent discovery that among their many neuron-like properties, EECs also express α-synuclein (Chandra et al., 2017). (Fig. 2) By virtue of their location at the interface of the gut lumen and enteric nerves, EECs may be subject to pathogen or toxin exposure that could affect α-synuclein. Should α-synuclein misfold in EECs, its transmission to α-synuclein-containing enteric neurons could be the first step in a prion-like cascade leading to PD (Chandra et al., 2017).

4. What causes abnormal α-synuclein in the gut?

Recently it has been demonstrated that the composition of the gut microbiome differs between healthy individuals and patients with PD (Hasegawa et al., 2015; Keshavarzian et al., 2015; Scheperjans et al., 2015; Unger et al., 2016). Moreover, the composition of the gut microbiota correlated with PD symptoms which have been associated with more extensive α-synuclein involvement in the enteric nervous system (Klingelhofer and Reichmann, 2015). However, it is not known if the changes in gut microbiota found in PD patients play a causative role in the pathogenesis of the disease or are simply a consequence of the disease. For example, altered intestinal motility, medications used to treat PD, or some other factor associated with the PD could influence gut microbe composition. Nevertheless, these findings raise the possibility that gut microbes affect PD pathogenesis.

This possibility was examined in a mouse model of PD in which microbiota from patients with PD transplanted into mice induced parkinsonian-like motor dysfunction (Sampson et al., 2016). Using germ-free mice, it was shown that gut bacterial colonization was necessary to induce α-synuclein pathology, neuroinflammation, and motor deficits. These changes appear to be mediated by microbial metabolites including short chain fatty acids. This study provides strong evidence that gut bacteria regulate α-synucleinopathy and the motor dysfunction of PD. Thus, some gut microbes may play a pathogenic role in PD.

However, how gut microbes and short chain fatty acids affect PD progression is not completely understood (Sampson et al., 2016; Unger et al., 2016). It is possible that EECs may be involved (Chandra et al., 2017) particularly since EECs are directly exposed to gut microbes and their metabolites and have been shown to express short chain fatty acid receptors 2 and 3 (FFAR2 and FFAR3) (Kaji et al., 2011; Nohr et al., 2013; Psichas et al., 2015; Sykaras et al., 2012).

5. Diagnosis of PD from the gut

Recognizing that Lewy pathology may be present in enteric nerves of PD patients, some investigators have begun to examine
pathological specimens for abnormal α-synuclein as a way to diagnosis PD. In a small study, 4 of 9 PD patients were found to have Lewy neurites in the submucosal plexus of the colon and 3 had detectable α-synuclein in the mucosa. In contrast, abnormal α-synuclein was undetectable in 10 control subjects (Poucle et al., 2012).

Alpha-synuclein is normally expressed in enteric neurons, therefore a reliable test must be able to distinguish abnormal (misfolded, aggregated or fibrillar) α-synuclein from normal α-synuclein. Intestinal specimens are typically analyzed microscopically using immunohistochemistry with antisera specific for phosphorylated or fibrillar α-synuclein or by recognizing α-synuclein aggregates in Lewy neurites (Schneider et al., 2016). Reliably detecting abnormal α-synuclein has not been easy (Corbille et al., 2016a) and even though it may be possible using a meticulous approach (Beach et al., 2016; Schneider et al., 2016), there are a number of unanswered questions. For example, it is not yet known what part of the intestine is most affected in PD. Many patients suffer from constipation suggesting that the colon is involved, but many patients also have nausea or gastroparesis that could be due to gastric or proximal intestine involvement raising the possibility that the upper gut may reveal neuropathology. Aggregated α-synuclein has been detected in stomach, proximal small intestine and colon of PD patients (Hilton et al., 2014; Lionnet et al., 2017), but in the absence of standardized techniques, there is not a uniformly agreed upon approach for assessing gastrointestinal tract involvement. It also is not known which region of the intestine might yield the greatest diagnostic sensitivity and specificity. To date, most attention has focused on enteric nerves of the submucosal or myenteric plexi. For practical reasons, submucosal samples are more readily available since submucosal nerves can be acquired by endoscopic biopsy (Lebouvier et al., 2010) but it is still unclear which nerves are more likely involved in PD. Identification of α-synuclein in EECs raises the possibility that EECs could be another site for evaluating changes in α-synuclein. By virtue of their location, EECs are readily accessible by endoscopic biopsy of the intestinal mucosa. Should EECs exhibit pathological changes in α-synuclein in patients with PD, EEC analysis could offer a tool for the diagnosis of the disease (Ruffmann and Parkinlen, 2016). Finally, alterations in α-synuclein abundance, phosphorylation, or aggregation are difficult to quantify. In the absence of a quantitative way to assess α-synuclein phosphorylation or aggregation, tests will be subject to observer interpretation.

Nevertheless, the substantial data suggesting that abnormal α-synuclein arises in the gut gives hope to the concept that detection in the gut could be used as an early predictor of clinical PD (Hilton et al., 2014; Shannon et al., 2012; Stokholm et al., 2016). However, even though gastrointestinal tract involvement is common in PD and abnormal α-synuclein is frequently detected in the intestine, considerable work remains to develop analytical methods for diagnosis or early detection of PD using intestinal sampling.

6. Targeting the gut to treat PD

The EEC-neural circuit offers an entry point from the lumen of the gut to the nervous system. Although this route may be hijacked by pathogens it also provides a pathway for potential treatments of the nervous system. The most obvious therapeutics that could be used to target EECs involve diet. Studies on dietary fat intake and PD have yielded conflicting results (Abbott et al., 2003; Anderson et al., 1999; Chen et al., 2003; de Lau et al., 2005; Logrosano et al., 1996; Miyake et al., 2010; Powers et al., 2009) although not all of these considered the type of ingested fats as polyunsaturated fatty acids have been associated with a lower risk of PD (Kamel et al., 2014).

Identification of gut microbial dysbiosis in patients with PD introduces intriguing possibilities for potential treatment. Experimental data suggest that microbes themselves affect the progression of α-synuclein pathology and that microbial metabolites such as short chain fatty acids may influence parkinsonian motor function. If similar microbial effects can be demonstrated in humans, it may be possible to treat PD patients with therapies that modify the gut microbiome or their metabolites to improve symptoms and possibly limit disease progression.

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