Experimental Neurology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review Article

Experimental animal models of Parkinson's disease: A transition from assessing symptomatology to α-synuclein targeted disease modification

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ARTICLE INFO

Keywords:
α-Synuclein
Parkinson's disease
Animal models
Lewy Body

ABSTRACT

With the understanding that α-synuclein plays a major role in the pathogenesis of Parkinson's disease (PD), novel animal models have been developed for conducting preclinical research in screening novel disease modifying therapies. Advancements in research techniques in α-synuclein targeted disease modification have utilised methods such as viral mediated expression of human α-synuclein, as well as the inoculation of pathogenic α-synuclein species from Lewy Bodies of PD patients, for accurately modelling progressive self-propagating neurodegeneration. In applying these cutting-edge research tools with sophisticated trial designs in preclinical drug trials, a useful platform has emerged for developing candidate agents with disease modifying actions, promising a greater chance of success for clinical translation. In this article, we describe the transition of well-established animal models of PD symptomatology to newly developed models of PD pathogenesis, with specific focus on methods of viral-mediated and inoculation of pathogenic α-synuclein, that aim to aid scientific translation of neuroprotective strategies.

1. Parkinson's disease pathogenesis

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting approximately 1% of the population over the age of 55 years, with the highest prevalence in ages of 85 years and over (De Rijk et al., 1997). PD patients display a clinical syndrome of motor symptoms (in particular bradykinesia, postural deficits and resting tremor) (Marsden, 1994), which may be preceded by a range of non-motor symptoms (including cognitive disturbance, sleep disturbance, anosmia and constipation) (Pont-Sunyer et al., 2015). These clinical manifestations of PD appear due to the extensive loss of dopaminergic neurons of the nigrostriatal pathway (Ehringer and Hornykiewicz, 1960), as well as dysfunction of neurons in dopaminergic, serotonergic, adrenergic and cholinergic neurotransmitter systems (Jellinger, 1991).

A major pathological hallmark of PD is the widespread expression of intraneuronal proteinaceous inclusions, known as Lewy Bodies (LBs) (Spillantini et al., 1998), found in the perikarya of neurons within the central and peripheral nervous systems. LBs are mainly composed of α-synuclein, a 14 kDa endogenous protein of 140 amino acid length. Autosomal dominant forms of PD are linked to missense mutations of the α-synuclein, specifically *p.A53T, p.A30P, p.E64K, p.H50Q, p.G51D* and *p.A53E* (Appel-Cresswell et al., 2013; Athanassiadou et al., 1999; Kruger et al., 1998; Lesage et al., 2013; Polymeropoulos et al., 1997; Puschmann et al., 2009; Zarranz et al., 2004) and also the multiplication and triplication of the gene locus for α-synuclein (SNCA) (Chartier-Harlin et al., 2004; Singleton et al., 2003).

In the last two decades, the α-synuclein protein has been proposed as the main component in mediating the progressive neurodegeneration in PD (Dehay and Fernagut, 2016; Recasens and Dehay, 2014). The pathological structure of α-synuclein is a misfolded conformation of an oligomeric or fibrillary nature that is of a specific phosphorylated form (phosphor-Ser129), as seen in PD patient LB formations (Fujiwara et al., 2002; Spillantini et al., 1997). Several studies have greatly impacted the field of PD pathogenesis. Braak et al. first hypothesised the progression of PD in distinct stages; beginning at the peripheral autonomic nervous system, a region where α-synuclein pathology was identified (Braak et al., 2006; Gelpi et al., 2014), in the anterior olfactory nucleus and dorsal motor nucleus of the glossopharyngeal and vagal nerves, gradually reaching the midbrain and cerebral cortex (Braak et al., 2003). Pathological transmission was later suggested between neurons through post-mortem analysis in PD patients, where LB formations were found in embryonic mesencephalic neuron grafts in the striatum that were implanted over a decade prior, supporting a host-to-graft transmission process (Kordower et al., 2008; Li et al., 2010). With the transmission of

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http://dx.doi.org/10.1016/j.expneurol.2017.07.020
Received 17 June 2017; Accepted 28 July 2017

α-synuclein-positive intracytoplasmic inclusions, the term 'prionoid' has been assigned, describing the pathogenic nature of intracellular mechanisms, including release into extracellular space and uptake into healthy neurons. Although mainly focused within dopaminergic neurons of the SN, this self-propagating process with the pathogenic α-synuclein acting as a template for misfolding (Brundin et al., 2008; Dehay et al., 2016a), can lead to a spread of neurodegeneration across interconnected brain regions causing disruption of intracellular processes (i.e. neurotransmission, mitochondrial structure and activity, dynamics and mitophagy, vesicular transport and protein degradation). The pathogenic α-synuclein seeding activity is supported by detection of monomeric, oligomeric and Ser129-phosphorylated α-synuclein forms in human plasma and cerebrospinal fluid (Borghi et al., 2000; El-Agnaf et al., 2003; Foulds et al., 2012; Mollenhauer et al., 2011; Tokuda et al., 2010). Moreover, further supportive evidence comes from the use of synthetic recombinant α-synuclein preformed fibrils (PFFs) inducing conformation changes of endogenous soluble α-synuclein to pathological species, subsequently leading to cellular dysfunction (Hansen et al., 2011; Luk et al., 2009; Volpicelli-Daley et al., 2011).

In recent years, the understanding of PD pathogenesis has been greatly enhanced with the development of advanced research tools, some taking the form of novel animal models (Angot et al., 2010; Dehay et al., 2016b; Olanow and Brundin, 2013). In this review, we provide an overview of the commonly used symptomatic animal models of PD and newly developed models of PD pathogenesis, of which the latter are expected to aid the translation of potential therapies with focus on disease-modifying mechanisms, paving the way for the identification of novel agents for treating neurological disorders.

2. Modelling clinical symptoms of PD

To date, neurotoxin based animal models have been well utilised in translational research for the development of symptomatic therapies in PD. The most commonly used neurotoxins include 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which are often administered in rodents and non-human primates (nhps), respectively, for inducing a parkinsonian state by causing severe loss of dopaminergic neurons.

In rats, the unilateral injection of 6-OHDA into the medial forebrain bundle causes rapid and extensive loss (> 90%) of dopaminergic neurons that is seen within 14 days (Grealish et al., 2008). The reproducibility and simplicity in quantifying asymmetric motor deficits, such as rotation and forelimb bias, following administration of dopaminergic agents are major advantages for the use of 6-OHDA-lesioned rats in translational research that have allowed for efficient screening of anti-parkinsonian compounds (Cannon and Greenamyre, 2010; Lane et al., 2006; Schwarting and Huston, 1996). In addition, the injection of 6-OHDA into the striatum permits for slower and less severe loss of dopaminergic nigrostriatal neurons (> 60–70%), modelling more progressive neuronal degeneration (Kirik et al., 1998; Sauer and Oertel, 1994).

The gold standard model of PD motor symptoms is produced by administration of MPTP into old world non-human primate (nhp) species (*Macaca mulatta* and *Macaca fascicularis*). With a closer resemblance to humans in physiology and brain anatomy, the PD motor features in monkeys that are induced following a regimen of MPTP injections (typically daily doses of 0.2–2 mg/kg for 2–3 weeks) gives an accurate model for advanced PD clinical motor signs (Bezard et al., 2001; Bezard et al., 2003; Meissner et al., 2003). The MPTP-treated nhp model is most commonly replicated after systemic administration of MPTP, which readily crosses the blood brain barrier, inducing a parkinsonian syndrome of prolonged stillness, bradykinesia, postural deficit and an overall reduction in movement (Crossman et al., 1985; Delong et al., 1985). After stabilisation of these behaviours where dopaminergic degeneration reaches > 90% in the substantia nigra pars compacta (SNc) (Bezard et al., 1997), subjective rating scales based on clinical scale criteria can be utilised to specifically assess movement range, posture, tremor, and a calculated score for overall disability (Ko et al., 2014). These translational tools are and have been instrumental for evaluating the efficacy of symptomatic therapies (Bastide et al., 2015; Johnston and Fox, 2015; Fox and Brotchie, 2010), which are effective in providing drug efficacy in proof-of-concept tests (Ko et al., 2014; Rylander et al., 2010). Furthermore, in recent years, sophisticated technology in high definition video recording of limb trajectory has been adapted to nhps for assessment of detailed kinematics in swing and gait cycles during freely moving tasks (Capogrosso et al., 2016; Yin et al., 2014). Such objective assessments of motor activity complement subjective behavioural ratings for detecting more subtle motor deficits, allowing for consistent endpoint assessments between MPTP-treated nhps and PD patients, in respective preclinical and clinical trials.

The MPTP-treated nhp model of PD offers further face and predictive validity (i.e. phenomenological similarities between behaviours and measurable treatment effects as seen in the human condition, respectively) with clear reversal of experimental parkinsonism following L-3,4-dihydroxyphenylalanine (L-DOPA) treatment. The maximum L-DOPA dose is individually titrated based on motor symptom severity and is commonly given during the stabilisation period of model replication to aid animal feeding. Daily L-DOPA treatment (in the range of 9–20 mg/kg) over 3–4 months can lead to the development of stable and reproducible dyskinesia, with chorea and dystonia individually scored based on severity (Fox et al., 2012). After L-DOPA priming in MPTP-treated nhps, PD disability cannot be completely reversed without the induction of dyskinesia, similar to that seen in PD patients. This has been utilised to provide clinically relevant data such as 'good on-time' (Ko et al., 2016; Pinna et al., 2016) allowing treatment strategies for dyskinesia to be thoroughly evaluated in dyskinetic MPTP-treated nhps. Investigations utilising this model also provide for addressing specific intracellular mechanisms using novel biotechnological approaches, such as gene therapy (e.g. modulation of regulators of G-protein signalling proteins (Gold et al., 2007)) or enzymes for dopamine synthesis (Jarraya et al., 2009), for testing potential therapies in modifying the expression of dyskinesia.

The use of MPTP-treated nhps is also a valuable translational research model for studying the non-motor symptoms of PD. For example, chronic low dose (CLD) MPTP administration can be used to induce cognitive deficits found in executive and attentional tasks, which are further worsened following L-DOPA treatment, as seen in PD patients (Gotham et al., 1988; Kulisevsky et al., 1996; Schneider et al., 2013). In modelling both cognitive deficits and subtle motor dysfunction in MPTP-treated nhps, the translational value allows for tests of single or combined drug treatment strategies that produce the most desired clinical outcome among the myriad of disease symptoms (Ko et al., 2016). Sleep disturbances seen in PD patients such as rapid eye movement sleep behaviour disorder (Postuma et al., 2009) can also be evaluated in MPTP-treated monkeys using continuous electroencephalography, which has been used to assess the effects of different pharmacological treatments on sleep disturbances (Barraud et al., 2009; Hyacinthe et al., 2014). While MPTP administration causes severe loss of dopaminergic neurons in the SNc, there is also a broad neurotoxic effect with loss of extra-nigral dopaminergic, serotoninergic and adrenergic neurons (Forno et al., 1986; Mitchell et al., 1985; Perez-Otano et al., 1991). Although this resembles some of the major neuropathological changes seen in PD patients, this animal model remains limited for studying disease pathogenesis due to lack of true LB pathology, albeit certain proteinaceous inclusions have been previously reported in MPTP-treated baboons (Kowall et al., 2000).

While neurotoxin based animal models serve for the development of symptomatic therapies, notably seen from the early success of deep brain stimulation in PD (Benazzouz et al., 1993), these symptomatic animal models remain limited for studying disease-modifying therapies due to the inability to accurately replicate the nature of PD pathogenesis i.e. progressive self-propagating neurodegeneration with