Published in final edited form as:
Mov Disord. 2013 January ; 28(1): 61–70. doi:10.1002/mds.25108.

Animal Models of Parkinson's Disease: limits and relevance to neuroprotection studies

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Summary

Over the last two decades significant strides has been made towards acquiring a better knowledge of both the aetiology and pathogenesis of Parkinson's disease (PD). Experimental models are of paramount importance to obtain greater insights into the pathogenesis of the disease. Thus far, neurotoxin-based animal models have been the most popular tools employed to produce selective neuronal death in both *in vitro* and *in vivo* systems. These models have been commonly referred to as the *pathogenic* models. The current trend in modelling PD revolves around what can be called the *disease gene-based* models, or *etiologic* models. The value of utilizing multiple models with different mechanism of insult rests on the premise that dopamine (DA) producing neurons die by stereotyped cascades that can be activated by a range of insults, from neurotoxins to down-regulation and overexpression of disease-related genes. In this position paper, we present the relevance of both pathogenic and etiologic models as well as the concept of clinically relevant designs that we argue should be utilized in the pre-clinical development phase of new neuroprotective therapies before embarking into clinical trials.

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Author contribution statement

EB, ZY, DK and MGS jointly performed the literature search and wrote the review.

Full financial disclosures of authors during the past year

EB is employed by INSERM (France), owns stocks in Motac Holding Ltd. (Manchester, UK), receives consultancy from Motac Neuroscience Ltd. (Manchester, UK), has received grants from the Agence Nationale de la Recherche (France), the Michael J. Fox Foundation for Parkinson Research (USA) and the European Commission and is a member of the scientific advisory board of the Michael J. Fox Foundation for Parkinson Research (USA), of the French Parkinson Association as well as from Parkinson's UK. During the past year DK acted as expert consultant for Amsterdam Molecular Therapeutics and AstraZeneca AB. DK is a co-inventor of a patent application on gene therapy for PD and receives financial compensation in the form of retainer fees, milestones and royalty payments. MGS is employed by the University of Cambridge and has been consultant for Noscira LTP in 2011. MGS is recipient of grants from parkinson's UK, The Alzheimer's Research UK, Michael J Fox Foundation, European Commission, Cure PSP USA and The Cambridge Newton Trust. During last year ZY consulted for Pfizer, Inc. and Millennium Pharmaceuticals, Inc. ZY received grants from Michael J Fox Foundation and NIH/NINDS.

Keywords

6-OHDA; MPTP; alpha-synuclein; LRRK2; neurodegeneration; dopamine; viral vectors

In contrast to other neurodegenerative conditions, there is relatively good symptomatic therapy for Parkinson's disease (PD). In early stages of the disease, in particular, dopaminergic medication can help sustain a good quality of life. However, as these treatments do not change the course of the disease, over years more and more patients enter into complication phase when the response profile worsens and the side effects of the medication starts to overweigh the benefits of the treatment. Currently, there is no proven therapy that can prevent cell death or slow its progression by restoring the normal function in sick neurons. Thus, interventions that can slow or halt the progression of PD remain a crucial unmet need. Our ability to deliver better therapeutic strategies to the clinics has been limited by the lack of in depth understanding about why and how neurodegenerative process begins and progresses in Parkinson's. Over the last two decades significant strides has been made towards acquiring a better knowledge of both the aetiology and pathogenesis of PD thanks to numerous elegant clinical and post-mortem studies and utilization of new *in vitro* and *in vivo* experimental models of the human disease. Despite these unquestionable advances made in the molecular and cellular biology of PD, which brought us closer than ever to being capable of unravelling the pathophysiological basis of PD, we still have gaps in our understanding of the mechanisms implicated in parkinsonian neurodegeneration that needs to be addressed.

Investigators in the field of Parkinson's rely heavily on the experimental models to obtain greater insights into the pathogenesis of the disease. Thus far, among the widely used animal models of PD, those that are based on administration of neurotoxins have been the most popular tools employed to produce selective neuronal death in both *in vitro* and *in vivo* systems. These models have been commonly referred to as the *pathogenic* models. The current trend in modelling PD revolves around what can be called the *disease gene-based* models, or *etiologic* models.

The value of utilizing multiple models with different mechanism of insult rests on the premise that dopamine (DA) producing neurons die by stereotyped cascades that can be activated by a range of insults, from neurotoxins to down-regulation and overexpression of disease-related genes or even manipulation of genes whose relevance to PD per se remains uncertain. In this position paper, we will briefly present the relevance of both pathogenic and etiologic models as well as the concept of clinically relevant designs that we argue should be utilized in the pre-clinical development phase of new therapies before embarking into clinical trials.

Neurotoxin models

Key neurotoxic models of PD include that produced by the toxin 6-hydroxydopamine (6-OHDA) most commonly used in rats but also in mice and marmosets, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is now almost exclusively used in mice and a variety of non-human primates. The administration of MPTP to various animals, i.e. monkeys, mice, cats, rats, guinea pigs, dogs, sheep and even frogs and goldfish, has however been shown to also cause parkinsonian-like motor disturbances [1–4].

Other less often utilized neurotoxins are rotenone, paraquat, isoquinoline derivatives and methamphetamine [5]. While it is important to cite the attempts to produce new models with the latter toxins, so far none has succeeded in generating a reliable and [4] new therapies.

Further validation work may in the future make them ready for more widespread use in preclinical drug testing, especially in the context of neuroprotection.

A number of reviews have described the pros and cons as well as the specifics of the several refinements of 6-OHDA and MPTP models [5–12]. While the molecular mechanisms of cell death have received much attention [11], the anatomo-pathological correlates are often less well known. Although the ability of these neurotoxins to induce marked, if not total, and long-lasting lesions of the nigrostriatal pathway is widely accepted, their ability to replicate the dorso-ventral gradient of striatal denervation in PD [13, 14] is still a matter of discussion despite clear experimental evidences. Discrete intrastriatal administrations of 6-OHDA in the rat can achieve such replication [15]. Striking examples of such preferential dorso-ventral striatal denervation have been produced with the MPTP in the marmoset [16], the African green monkey [17] and the macaque monkeys [18–20] suggesting that chronic exposures consistently allow to mimic the human PD specific pattern of nigrostriatal lesion while earlier intracarotidian and acute regimen did not.

In addition to degeneration of the nigrostriatal dopaminergic pathway, PD is characterized by the involvement of a variety of neuronal systems, causing a multiple neuromediator dysfunction that accounts for the complex patterns of behavioral deficits. Parkinsonian neurodegeneration is known to affect the dopaminergic mesocorticolimbic system, the locus ceruleus rich in noradrenaline (NA) producing neurons and motor vagal nucleus, the serotonergic raphe nuclei, the cholinergic nucleus basalis of Meynert, pedunculopontine nucleus pars compacta, Westphal-Edinger nucleus, and many peptidergic brainstem nuclei. A classic rebuttal to the neurotoxin models is their supposed specificity for the dopaminergic system. To ensure such “specificity”, 6-OHDA is classically injected after pre-treatment with desipramine, a blocker of the uptake by noradrenagergic neurons, and pargyline, an irreversible monoamine oxidase B inhibitor. In our experience, however, the protection of the NA system with desipramine is rather variable. So in the absence of it, there is a consistent NA lesion concomitant with DA lesion. While in its presence, the outcome could vary from near complete preservation of the NA projections to the forebrain to no apparent protection. Often the pre-treatment with citalopram, a blocker of the uptake by serotonergic neurons is used to protect the projections from the raphe nucleus [12]. What these pre-treatments suggest is that 6-OHDA could kill the noradrenergic and serotonergic neurons. In fact, bilateral systemic administration of 6-OHDA without desipramine or citalopram leads to multisystemic lesions mimicking the best PD pathology with more complex phenotype than the drug-induced rotations typically studied in unilaterally lesioned animals. MPTP-induced toxicity as well is often presented as being “specific” for the dopamine neurons, although it is well known that MPTP induces multisystemic lesions. Original work from Hornykiewicz in MPTP-treated macaques showed that all three major brain monoamine neuron were sensitive to the neurotoxin [21]. In addition, each brain monoaminergic system had a characteristic regional pattern of MPTP-induced denervation. As expected, the most significant alterations were found within the nigrostriatal dopaminergic system, i.e. profound DA loss in caudate nucleus, putamen and substantia nigra. However, many extrastriatal regions of the subcortex and brainstem also suffered significant loss of DA, with NA loss in the regionally subdivided brainstem being less widespread, and the serotonin levels least affected. It was thus demonstrated that in the rhesus monkey MPTP mimicked, in addition to the profound striatal DA loss, some of the extrastriatal DA, NA and serotonin changes often seen in PD brains [21]. Since then several evidences have been accumulated showing such multisystemic lesions affecting the whole brain [22], the brain stem, the spinal cord [23] and the enteric nervous system [24].

Besides the progressive cell loss, the pathological hallmark of PD is the presence of intraneuronal proteinacious cytoplasmic inclusions, named Lewy Bodies (LB). These

inclusions are a result of intracellular deposition of proteins and lipids that were traditionally stained with hematoxylin/eosin. Biochemical and histological analyses have shown that α-synuclein is a major protein component of LB where it forms the chracteristic fibrillar structure [25], thereby linking familial and idiopathic forms of PD. The significance of neuronal inclusions in the disease process – i.e. whether they reflect a neuroprotective vs neurotoxic process – as well as the mechanisms responsible for the formation of inclusions remain unknown, although formation of oligomers has clearly been shown to be toxic in the *in vitro* settings. The construct validity of the neurotoxin models would be dramatically increased if such LBs were observed in these models. Although no LB as yet been reported in neurotoxin-based models despite investigations sometimes performed up to 10 years after MPTP insult [26], various MPTP intoxication regimens are reported to affect α-synuclein levels and aggregation. Indeed, α-synuclein aggregates have been observed in a chronic MPTP model [27]. Such phenomenon has been better documented in the MPTP monkey models in which a clear relationship between dopaminergic cell loss, α-synuclein upregulation, neuritic α-synuclein pathology and α-synuclein aggregation has been established [28–30]. Interestingly, such changes occurred mostly in neuromelanin-positive neurons [30]. This observation suggests a possible specificity of the primate DA neuron physiology compared to rodents. Oxidation of DA to aminochrome is a normal process where aminochrome polymerization is followed by neuromelanin formation [31]. Neuromelanin is a pigment that accumulates over time in the SNc dopaminergic neurons of macaques but not in rodents. Melanized neurons are more susceptible to neurodegeneration than non-melanized both in PD patients [32] and in MPTP-treated primates [30, 33]. Such susceptibility to degeneration might be caused, at least in part, by possible self-toxicity of neuromelanin or its intermediates, such as its ability to trigger inflammatory reactions [34]. In addition, the obvious life span differences across species might play a role as well. That primates, a long life span species, are unique in their expression of neuromelanin suggest that maybe only primate dopamine neurons are susceptible enough to various insults to degenerate and that they lack compensatory mechanisms present in rodent dopamine neurons.

Transgenic alpha synuclein models

Alpha-synuclein is the main component of the filaments that form the Lewy bodies, the characteristic inclusions present in PD and other alpha-synucleinopathies [35, 36]. Moreover, rare missense mutations and more common duplication and triplication of the α-synuclein gene have been identified as the cause of familial forms of PD [37, 38]. These findings have shown that changes in the expression of α-synuclein level can cause disease and have granted the possibility to produce transgenic mouse models of alpha-synucleinopathies by overexpressing mutant or wild type alpha-synuclein.

Several transgenic mouse models have been produced and various promoters have been used to drive the expression of the transgene leading to different results. The use of pan neuronal-promoters such as Thy1, PDGF and PRION promoters has given robust expression in many areas of the brain and in some cases in the spinal cord while expression of the transgene has been inconsistent in the substantia nigra [39–41]. The reasons for this are not clear. This brain area seems to have a tight control on the amount of the transgenic α-synuclein produced. This is apparent in some transgenic mice where the protein is expressed under the control of the TH promoter specifically in catecholaminergic neurons. In these mouse models, the transgene expression is controlled. In fact the transgene expression does not increase in homozygous mice compared to heterozygous littermates as observed in mice expressing 1-119 truncated α-synuclein [42] or in 1-120 mice (Spillantini et al. unpublished observation). It is not clear whether this control is present also in mice expressing the full-length protein. In one of the first mouse model to be produced [39] the transgene was expressed under the

PDGF promoter that led to a wide transgene expression in the brain with α-synuclein aggregates in both neuronal cytoplasm, some nuclei and also in glial cells. In transgenic mouse models where the Thy 1 promoter was used a more widespread neuronal expression of the protein than the PDGF promoter was observed and no consistent glial accumulation reported [41, 43–45]. In several transgenic mouse lines α-synuclein accumulation in the brain and spinal cord has been found, however no model so far has shown the full features expected for a proper PD model, i.e. α-synuclein aggregation, DA reduction and progressive dopaminergic cell death. Some mice show α-synuclein aggregation [39, 40, 46–48] and no cell death while others show cell death but no protein aggregation [49]. Some of the transgenic mice produced expressing full-length α-synuclein with the A53T or A30P mutations show abnormal motor behaviour associated with granular or filamentous material. However, in some cases these alterations appear to be mainly linked to the pathology in the spinal cord and not dysfunction of the substantia nigra [40, 50]. In order to have a consistent expression in the substantia nigra, several groups have specifically expressed the transgene into dopaminergic cells by using the TH promoter [43–45, 48, 49, 51, 52]. Mice expressing full-length wild-type α-synuclein in TH neurons have shown in some cases neuronal accumulation but no cell death [52]. On the contrary, mice expressing full-length α-synuclein with the A30P and A53T mutations have shown reduction of striatal DA and motor impairment but no α-synuclein aggregation [44, 49].

The formation of α-synuclein aggregates is one of the requirements for a model of PD but the presence of aggregates is quite inconsistent in the various modes. To promote and speed up α-synuclein aggregation, the use of carboxy-terminally truncated protein was introduced because it is known that α-synuclein carboxy-terminal part is inhibitory for aggregation [53, 54] and by removing it could speed up the aggregation process in a short-lived mouse brain. Furthermore, truncated α-synuclein has been found in brain extracts of PD and dementia with Lewy bodies patients [55–59]. Several groups have produced transgenic mice expressing truncated α-synuclein [42, 48, 52]. We have produced a transgenic mouse expressing truncated 1-120 α-synuclein under the control of the TH promoter in a C57Bl6 background with or without the endogenous protein that has been reported to be inhibitory for aggregation [54, 58, 60]. The lack of neuronal cell death and the presence of alpha-synuclein aggregation has prompted to consider the alpha-synuclein transgenic mice a model for the early stages of PD, by investigating what goes on in these mice could shed light on the early stages of the pathology. Indeed, studies from various groups in different alpha-synuclein transgenic and knock out mice as well as in cellular models are now coming together and point to the synapse, where alpha-synuclein is physiologically present, as the first place of alpha-synuclein-related dysfunction [61–68]. In the TH 1-120 α-synuclein transgenic mice we find accumulation of α-synuclein in cell bodies and neurites with a progressive degeneration of the latter [48]. Interestingly, in this mouse line we have identified the presence of reduced DA release in both striatum and hippocampus [65]. Several studies using both transgenic α-synuclein mice and cells in culture, as well as mice where α-synuclein has been knocked out, have shown a consistent defect at the synapse [65, 67, 68] and in the 1-120 α-synuclein transgenic mice this is associated with a redistribution of the SNARE complex, involved in exocytosis as well as of the dopamine transporter (DAT) have been found [65, 69]. Interestingly in these 1-120 mice synaptic aggregates of α-synuclein are co-localized with SNARE proteins and DAT molecules. All these synaptic alterations in the absence of cell death point to a “dying back” mechanism for PD with the abnormalities at the synapse slowly leading to formation of LB’s and neuronal death. Indeed, like in the 1-120 α-synuclein transgenic mice, in patients with Parkinson’s disease and Dementia with Lewy bodies α-synuclein accumulations are present at the synaptic level [65, 70] and in PD patients alterations in the sympathetic innervation of the heart start at the terminals [71]. Lewy bodies appear to be the result and indicator of a dynamic process going on in the neuron where they are localized [72]. Although in the majority of cases α-synuclein transgenic mice do not

appear to go all the way to show dopaminergic neuronal death, they have indicated how the pathological process could start confirming previous suggestion [73] that alterations in DA handling start very early in the pathogenic process. The study of α-synuclein transgenic mice with the recent observation in PD patients that only 30% of dopaminergic neurons are dead at presentation of motor symptoms [74, 75] indicates that intervening on the synaptic deficit could prevent cell death and full development of the disease.

Genetic LRRK2 models

Identification of LRRK2 (Leucine-rich repeat kinase 2) mutations as the genetic cause for *PARK8* [76, 77] has triggered another wave of PD model generation. LRRK2 encodes a large complex protein consisting of multiple conserved domains such as N-terminal ankyrin repeat, Leucine-rich repeat domain, ROC (GTPase) domain, MAPKKK-like kinase domain, and C-terminal WD40 domain. At least 6 mutations in LRRK2, G2019S, R1441C/G/H, I2020T and Y1699C, are believed to be pathogenic. G2019S mutation alone accounts for the most common inherited form of PD [78, 79] (~4%). In fact, mutations of LRRK2 are also linked to some sporadic forms and the clinical symptoms and neuropathology of LRRK2-associated PD are indistinguishable from idiopathic PD [76, 77]. The autosomal dominant transmission of LRRK2 mutations makes transgenic expression of pathogenic LRRK2 species suitable for modelling disease process in PD. Indeed, various invertebrate transgenic models producing LRRK2 PD mutants were quickly reported after the discovery of LRRK2 mutations in PD; the phenotypes of the models range from no change to apparent neuronal loss or deficits in DA systems and motor behaviour [80]. Certain lines exhibiting PD-related pathologies were reportedly used to evaluate LRRK2 kinase inhibitors in neuroprotection, revealing the potential value of the invertebrate LRRK2 models in drug screening [81].

Previous studies show that some, if not all, PD-linked mutations of LRRK2 cause enhanced kinase activity, which appears to be associated with increased cytotoxicity in cell culture [82, 83]. The evidence led to a hypothesis that the gain-of-function in kinase activity associated with LRRK2 pathogenic mutations is attributable to the neurotoxicity in PD. Over the past years, several groups have reported LRRK2 transgenic (including BAC-mediated approach) and knock-in mice producing pathogenic mutations in LRRK2 including G2019S or R1441C/G [84–88]. None of the transgenic or knock-in mice have however developed robust neurodegeneration or alpha-syn accumulation in the brain, which limit their application in mechanistic study of neurotoxicity and Lewy body formation as well as the development of neuroprotective strategies. Despite the lack of substantial neurodegenerative process and clinical syndromes of PD, certain PD-related phenotypes emerge as common among those models such as impairment of striatal DA transmission and abnormal phospho-tau levels [89]. In addition, the BAC transgenic LRRK2-R1441G mice show age-dependent locomotor deficits [88]; similarly, a fraction of (15%) BAC transgenic LRRK2-G2019S mice have late-onset (>20 months) motor behavioural abnormality (J. Wang and Z. Yue, unpublished observations). A most recent study reports a new transgenic line expressing LRRK2-G2019S that exhibits late-onset, modest loss of DA neurons at SNpc [90]. However, it is unclear at this stage whether the locomotor abnormality and neuronal loss are relevant to etiology of PD.

How do we evaluate the current LRRK2-G2019S or R1441C/G transgenic mice that are “imperfect” for modelling PD? First of all, these models are useful for understanding early pathogenic events in PD. Current evidence suggests that the striatal DA abnormality often precedes the frank motor function deficits in G2019S PD patients and perhaps loss of the dopaminergic neurons. Therefore, the LRRK2 rodent models may recapitulate the specific disease stage that present an early pathological alteration prior to the loss of nigral neurons. Second, the LRRK2 models can be used to explore the interactions between genetic risk and

environmental factors that underlie the PD etiology. The incomplete penetrance of the common mutation G2019S implicates significant contribution of additional factors (environmental and/or genetic) to the disease onset. For example, lack of exposure to certain disease-related environmental context may explain the absence of substantial PD symptom and pathologies in LRRK2 models that are housed in pathogen-free facilities. Recent evidence reveals a role of LRRK2 in inflammatory pathway [91]; microbial toxins that induce immune response may ultimately trigger the disease onset in the CNS expressing LRRK2 PD mutations with heightened kinase activity. Third, in terms of application of the LRRK2 models in pre-clinical research, we propose that some LRRK2 models should be valuable in aiding target identification and LRRK2 inhibitor evaluation *in vivo* [89]. With increasing number of LRRK2 kinase inhibitors identified, suitable mouse model(s) that have robust PD-related symptoms and pathologies are highly desirable for the assessment of the inhibitor efficacy animal models. The HSV or adenovirus-mediated LRRK2 rodent models may provide promising neurodegenerative models for the task likely in part due to their nature in promoting inflammatory reaction [92]. However, the overall high variability of the results may limit their general application. Therefore, genetic models developing robust, tractable and reproducible PD-related phenotypes are ideal. The following potential endpoints in evaluating the LRRK2 inhibitors in available genetic LRRK2 models can be considered. First, inhibition of LRRK2 kinase activity can be evaluated by assaying LRRK2 autophosphorylation and phosphorylation of LRRK2 substrates via phospho-specific antibody detection. In BAC transgenic wild-type LRRK2 (LRRK2-Wt) and LRRK2-G2019S mice, the kinase activity of brain LRRK2-G2019S protein is 2–3 fold higher than that of wild type [88]. These models are potentially useful to test the idea that reducing the aberrantly elevated kinase activity in G2019S to a non-toxic level (without complete inhibition of LRRK2 kinase activity) is desirable for the prevention of neuropathology in G2019S mice. Second, reversal of impaired striatal DA transmission (and levels) and tau pathology, the two common disease-related features in LRRK2 genetic models, can be employed as primary neuropathology endpoints, and alleviation of motor deficits in the BAC mice as potential behavioral endpoint. Furthermore, it would be ideal to test at least two models to have confidence in their inhibitory activity and neuroprotective function in vivo.

In summary, previous and ongoing investigation of these models has begun to shed light on LRRK2 cellular functions and pathogenic pathways. Continuous exploration of the existing and incoming new LRRK2 models will provide critical information for drug target validation and novel endpoint identification in the development of therapeutic strategies.

Viral vector-mediated modelling

Development of recombinant viral vectors for *in vivo* transfer of transgenes of interest has opened up a new possibility to model diseases in the central nervous system. Especially the adeno-associated virus (AAV) and lentiviral (LV) vectors have been successfully implemented as effective gene transfer tools with long lasting expression in the brain. The two vector systems show several differences in their biology, e.g., single-stranded DNA versus RNA as genetic material, or episomal expression versus integration into host genome. However, for purposes of gene transfer to the brain to model PD in animals, they both are very useful. On a more practical level what may influence the use of one or the other vector system as the delivery tool is the different capacities to carry transgenes and their affinity to transduce various neuronal cell populations in the brain.

Optimization of production and purification methods for AAV vectors yielding high titre vector preparation with capacity to transduction large numbers of cells in the brain and sustain expression over long term, as well as the ability of these cells to transduce nigral

dopamine neurons with very high efficiency, made AAV the vector of choice for delivery of disease causing genes to the nigral neurons [93]. After the identification of the point mutations in the α-synuclein gene, serotype 2 recombinant AAV vectors encoding for the wild-type or the mutated forms of human α-synuclein were used to transduce the nigral dopamine neurons in the rat [94, 95] and later in the non human primate [96]. These studies not only established that the AAV vectors could be used to deliver disease causing mutants to model PD, but also that modeling genetic aspects of PD was possible in species other than rodents. In parallel to the studies using AAV vectors, essentially the same results were obtained with LV vectors [97, 98]. Collectively, the main observation made in these studies was that overexpression of the human α-synuclein in nigral neurons led to neurodegeneration in the substantia nigra, a concomitant loss of fiber terminals in the striatum and reduction in tissue DA content. Importantly, these neuropathological changes were associated with abnormal accumulation of α-synuclein protein in the cell body and terminals of the affected cells and formation of dystrophic fibers resembling Lewy neurites, as seen in the parkinsonian brain. Contrary to neurotoxic models, which induce an acute lesion of the cells and fibers, the neuropathology in this model developed over several weeks.

In the rat brain, expression of wild-type or the A53T mutant form of α-synuclein appeared to result in similar end points, although the time course for the A53T variant could be faster [99]. Interestingly, in the marmoset brain, the toxicity of human wild-type α-synuclein is much less than the A53T variant [100]. Similarly, overexpression of the rat α-synuclein in the rat brain did not yield toxicity [98], suggesting that the rodent and primate α-synuclein proteins may have differential toxicity and this may be dependent on the species specific properties of the DA neurons. Moreover, not all cell types appear to be equally vulnerable to the α-synuclein overexpression, as dopaminergic neurons in the ventral tegmental area or the GABAergic neurons located in the pars reticulata of substantia nigra did not seem to degenerate after transduction with the same viral vectors [98, 101].

Main advantages of using viral vectors to generate models of PD include (1) the ability to use otherwise wild-type animals as hosts which is then not limited to mice; (2) to induce the expression of the transgene in a targeted manner in the cell populations to be studied and in adult animals, avoiding the confounding effects of developmental compensation; and (3) the possibility to study unilaterally transduced animals, where the contralateral side could be used as an internal control and lateralized motor tests could be used to assess the motor deficits in the affected and the contralateral limbs separately. There are a number of shortcomings that should be noted too. The transduction is done in each animal individually via a surgical intervention and direct injection of the vector in the target area in the brain. This results in variable degree of expression in different cells in the same brain and different levels of expression between subjects making the model rather heterogeneous. This variability impacts on the degree by which animals followed in a study would show behavioural impairments. Typically 25–30% of animals in a cohort injected with AAV vectors encoding α-synuclein would develop deficits [95], although more recent data suggest this fraction could be increased with use of higher titer vectors and different serotypes than AAV2 [102, 103].

Despite the variability in the neurodegeneration in the nigra (typically between 30–80% TH-positive cell loss), the viral vector based models have already been used to test strategies for neuroprotection. One of the key questions was the efficacy of neurotrophic factors (in particular the GDNF family of neurotrophic factors), which were entering in clinical trials in PD patients and had shown remarkable efficacy in neurotoxin based models both in rodents and primates (see for review [104]). Thus, Lo Bianco and colleagues tested the efficacy of LV-GDNF in the α-synuclein overexpression model and found, surprisingly that GDNF had no detectable effect in this model [105]. These findings were later confirmed and extended in a

recent study [106]. At present it is not clear how the discrepancy between the two models could be interpreted. However, positive results obtained after over-expression of parkin (another PD related gene) in both models [107, 108] suggests that this was not simply due to possible artifacts related to the over-expression of proteins using viral vectors but more specifically the mechanism of neuroprotective action of GDNF in relation to the insult generated by increased α-synuclein load in DA neurons.

Clinically-relevant experimental designs

The literature is rich in both small molecule and other drug candidates holding some promise as neuroprotectants in PD. According to the classic operational definition of neuroprotection, it would be any intervention that favourably influences the disease process or underlying pathogenesis to produce enduring benefits for patients. This definition comprises related terms such as neurorescue and neurorestoration. A likely process for deciding upon the push towards a clinical trial involves reaching predefined evaluation criteria typically defined as action upon an accepted primary mechanism of cell death in PD, consistency of preclinical data, blood brain barrier penetration, safety/tolerability ratio and relevant animal model efficacy.

It is at this point of compound selection where the complications arise. Today, the selection of such a candidate would primarily rely on data from MPTP-induced or other neurotoxin-induced models of PD, since genetic animal models of PD and other neurodegenerative disorders, which may have related pathogenic mechanisms, are just beginning to be used in preclinical validation of drugs of interest. Our goal here is not to compare the relative merits of the available models, but to simply raise the issue of the experimental design that has led to the demonstration of efficacy of given compounds in these models. While PD is a progressive neurodegenerative disorder, most agents have been tested in acute or semi-chronic neurotoxin-induced models and have even been adminsitered prior to lesion. While PD patients are likely to receive a neuroprotective agent following diagnosis, i.e. when the extent of dopamine neuron degeneration is already approximately 50%, drug candidates are tested in association with, or even weeks ***before***, neurotoxin administration. The relevance of such administration protocols with regard to the natural progression of PD is therefore questionable.

Thus, there is a crucial need for testing drug candidates in clinically driven experiments that should ideally fulfil four criteria: First, the chosen animal model(s) should recapitulate features of sporadic PD including its progressive nature (this eliminates all acute models, but for initial screening). Second, administration of the drug candidate should begin after the neurodegeneration is induced, ideally from a pre-defined level of DA neuronal loss in order to mimic what would happen in patients. Third, demonstration of efficacy should be provided in both a pathogenic and a etiologic model, as they are likely to involve different stereotyped cascades leading to cell death in clinical PD. Fourth, final proof of efficacy should be obtained in non human primate models (again, pathogenic and etiologic), since it is likely that complex cell death mechanisms may differ in rodents and primates. Such progressive non human primate models that mimic the progression of dopamine neuronal death in PD already exist using either neurotoxins [18] or viral vector mediated delivery of disease-relevant genes or shRNA of such genes [96, 100] (Bezard & Dehay, unpublished data).

Unfortunately, academic centres do not usually have sufficient funding to test candidate drugs in such expensive models and seldom have the possibility to test both pathogenic and etiologic models. The pharmaceutical industry would typically have the required funding to carry out such studies, but has not yet accepted the need approach drug development using models based on multiple mechanisms. In addition, the industry does not necessarily publish

negative data or results that may have deleterious affects to their drug development time lines. As a consequence, only a small fraction of drug candidates have been tested in such chronic pathogenic models with administration starting only once DA denervation is at clinically relevant levels (30–50% loss), i.e. in a clinically driven experimental design. Using such design in pathogenic models, few drug candidates have shown efficacy, i.e. the capability to delay the appearance of clinical symptoms associated with a rescue/protection of the remaining dopaminergic neurons. Some drug candidates previously shown to be potent in co- or pre-treated acute neurotoxin-induced models have failed or even displayed a deleterious effect in chronic models. For instance, the tetracycline antibiotic minocycline, one of the 12 selected drug candidates by the Committee to Identify Neuroprotective Agents in Parkinson's disease (CINAPS) of the National Institute of Neurologic Disorders and Stroke (NINDS) [109], proved to be deleterious in two different chronic models, including the progressive non-human primate model of PD [110, 111].

Conclusion

The proliferation of candidate drugs may bring more angst than excitement when the time comes to choose the few ones to be tested in PD patients. Although it is impossible, in essence, to remove all doubt prior to testing a drug in patients (c.f. the concept of clinical equipoise)[112], our obligation is to establish the most sound and clinically-relevant preclinical validation new drugs prior to testing in humans. In this vein, it is our opinion that there is no "best model of PD," as none is a true pathocopy of the human condition; these models are only approximations, each possibly holding a certain degree of relevance. Thus, to increase the chance of fruitful preclinical investigations, translational researchers should first know the strengths and the weaknesses of each model, second, select models whose characteristics are most suitable for addressing the experimental question, and third, apply clinically-relevant designs.

Acknowledgments

The Université Victor-Segalen Bordeaux 2 and the Centre National de la Recherche Scientifique provided the infrastructural support to EB. EB is supported by Agence Nationale de la Recherche grants (ANR-07-JCJC-0090; ANR-08-MNP-018 ; ANR-07-MNP-Trafinlid). DK is supported by grants from the Swedish Research Council (Project Grant No. 2009-2318, Senior Researcher Position, Bagadilico Linné Program No. 2007-8626 and MultiPark Strategic Research Environment), European Research Council (TreatPD, No. 242932) and the Michael J Fox Foundation. ZY is supported by NIH/NINDS grants NS060809–01, NS072359–01 and Michael J. Fox Foundation for Parkinson's Research. MGS is supported by the Parkinson's UK and Michael J Fox Foundation.

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