

Review Article

Limitations of Animal Models of Parkinson's Disease

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Most cases of Parkinson's disease (PD) are sporadic. When choosing an animal model for idiopathic PD, one must consider the extent of similarity or divergence between the physiology, anatomy, behavior, and regulation of gene expression between humans and the animal. Rodents and nonhuman primates are used most frequently in PD research because when a Parkinsonian state is induced, they mimic many aspects of idiopathic PD. These models have been useful in our understanding of the etiology of the disease and provide a means for testing new treatments. However, the current animal models often fall short in replicating the true pathophysiology occurring in idiopathic PD, and thus results from animal models often do not translate to the clinic. In this paper we will explain the limitations of animal models of PD and why their use is inappropriate for the study of some aspects of PD.

1. Introduction

The goal of most studies focused on understanding idiopathic PD is to identify the triggers and the mechanisms involved in the progressive neurodegeneration associated with the disease, to design treatments for the symptoms and to develop strategies to slow or stop neurodegeneration. Ideally, a model of idiopathic PD would be progressive in nature allowing the characterization of mechanistic changes in the brain and the onset of symptoms with time. Such a model would provide an opportunity to intervene as the disease progressed. Toxin-based models fall short in this regard since their acute nature, a single or a few injections given over a short period of time followed by rapid or immediate onset of symptoms, limits their usefulness. In addition, the best animal models should mimic the pathophysiology of the disease including the formation of alpha, synuclein, containing inclusions (Lewy bodies), the loss of neurons in the substantia nigra pars compacta (SNpc), and behavioral symptoms that arise during the course of the disease [1]. Taking these important issues into consideration, the best animal models for PD would provide a gradual onset of pathophysiological symptoms and only after manifestation of symptoms would a drug or neuroprotective agent be administered to test for effectiveness [2]. When a genetic model is used to study PD,

treatment could be administered prior to the onset of the symptoms. This clinically driven approach that mimics the development of the disease in patients is rarely used in animal studies although there are a few exceptions [3, 4].

A widerange of models have been used to study PD from the small evolutionarily remote single cell yeast to the large evolutionarily similar nonhuman primate. Yeast [5], worms [6], and fruit flies [7] are useful for studying fundamental cellular processes involved with PD, such as apoptosis, autophagy, oxidative stress, protein misfolding and degradation, vesicle-mediated transport, and determining the function of proteins. Some of the factors known to be involved with PD have no known homologs in the smaller eukaryotes, nevertheless expression of human genes in these organisms has been useful in partially elucidating the role of the proteins. Whether it is possible to entirely determine the function of proteins using heterologous expression remains unclear particularly because important protein-protein interactions may not be evolutionarily conserved. In addition, these small animal models cannot be used to study many of the clinical manifestations of the disease [8], nor can yeast, worms, or fruit flies replicate the loss of neurons in the brain [7].

Throughout the years of PD research, rodents have been widely used to study the disease because they are readily

available, genetically malleable, and relatively low cost as compared to larger animals. There are several excellent studies that have used dogs, cats and nonhuman primates for PD studies, but the ethical concerns and costs of such studies have limited their utility. Because of the widespread use of rodent models and their similarities to humans, they will be the focus of this paper.

2. Modeling PD in Rodents Using Environmental Toxins

To the best of our knowledge, PD does not appear to develop naturally in any animals except humans. The standard models for PD are designed to produce nigrostriatal dopaminergic lesions usually with 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat or, rotenone [9–12]. Most of these models inhibit mitochondrial function and/or create reactive oxygen species, but none of them completely reproduces the clinical symptoms and pathology of PD seen in humans [12]. Although these models are used extensively to study the mechanism of disease onset and progression and the efficacy of therapeutic treatments, the results obtained using these models rarely translate to the clinic successfully [13, 14]. Part of the problem with most of the toxin models is their acute nature, which is completely different from the insidious progression of PD observed in patients. Compensatory changes may arise in patients over the course of the disease that would not have an opportunity to occur in the acute animal models. In addition, PD occurs most frequently in elderly patients, usually around the age of 60 or older. Unfortunately, most rodent models do not use older animals because of the inconvenience and cost of housing the animals for an extended period of time. In addition, a closer look at the differences in behavior, physiology, and gene expression between rodents and humans as described below partially reveals why the animal studies do not translate well to clinical studies.

3. Can Genetic Models Be Used to Study Idiopathic PD?

Some recently developed models for studying idiopathic PD have taken advantage of either genes known to play a role in PD from familial studies or genes whose expression is significantly altered in PD patients compared to controls. Models using inherited mutated familial genes are designed to create null mutations of recessive genes or to express additional copies of dominant genes in mice. The genetic models have recently been reviewed elsewhere and therefore are not described in detail in this paper except for a few of the most promising recently developed models [15, 16]. One of the mouse models expresses the human α -synuclein gene with two mutations (A30P/A53T) that produce dominantly inherited forms of PD under the control of the tyrosine hydroxylase (TH) promoter that restricts expression to catecholaminergic neurons [17]. The benefit of this genetic model is that an age-dependent loss of TH-positive neurons

in the SNpc is observed along with a decline in motor activity. No Lewy bodies are observed in this model however. In addition, there are no known familial cases of PD in which both mutations have arisen in the α -synuclein gene, thus the relevance of the model has been questioned [15]. In another approach to developing genetic models, transgenic mice were created that used the TH promoter to overexpress truncated forms of α -synuclein [18, 19] that had been shown to be pathologically relevant to PD [20–23]. One of these models showed selective nigral DA neuron degeneration and impaired locomotive function that was reversed by L-DOPA treatment similar to PD in humans [19]. Unfortunately, the loss of neurons in this model was not progressive and occurred during embryogenesis thus substantially reducing the value of this model for collecting information pertinent to PD in humans. An alternative approach for overexpressing α -synuclein is stereotactic injection of the gene carried on viral vectors into the SN which produced rodents with DA neuron degeneration [24–26]. Despite the availability of numerous α -synuclein-based genetic models of PD, only the mouse prion promoter A53T α -synuclein transgenic mouse shows the same α -synuclein pathology and age-dependent neurodegeneration that is observed in humans [27–30]. One of the most recent additions to the selection of α -synuclein models of PD is a transgenic mouse that expresses the wild type gene with the regulated tetracycline (tet) system [31]. In this model, loss of neurons in the SN, progressive motor decline, hippocampal pathology and cognitive impairment were observed, but there were no fibrillary inclusions. This model has provided one very important piece of information in understanding PD, however. The ability to terminate expression allowed the investigators to conclude that continual expression of α -synuclein was required for disease progression [31].

There has been much more limited success in producing a genetic model of PD using several autosomal recessive genes including Parkin, PINK1 and DJ-1 (reviewed in [15]). Recently more attention has been directed at LRRK2 since mutations in this gene account for 5%–6% of patients with familial PD and 1%–3% of sporadic PD patients [32, 33]. Unfortunately, most of the transgenic mice that express wild type or mutated versions of LRRK2 exhibit minimal or no neurodegeneration [16]. This is also true of the wild type and mutant LRRK2 bacterial artificial chromosome (BAC) transgenic mice [34]. Despite this caveat, an advantage of the LRRK2 BAC transgenic mice is that they exhibit a progressive age-dependent motor deficit that responds to L-Dopa and apomorphine treatment [35].

Promising alternatives to the strict genetic models are genetic models that are additionally exposed to toxins such as MPTP. Since the development of PD may be caused by exposure to environmental toxins or heavy metals combined with a genetic vulnerability, these newer combination models could prove to be extremely beneficial for studying PD. In addition, some of the more refined genetic models of PD alter the expression of genes of interest in specific regions of the brain or specifically in neurons. One of the most promising models in this category is the MitoPark mouse [36]. In this model the mitochondrial transcription factor TFAM may

be conditionally inhibited in dopamine neurons. MitoPark mice exhibit motor impairment, reduced dopamine in the striatum and loss of dopamine neurons particularly in the SNpc. Intracellular aggregates form in the brain of MitoPark mice, but unfortunately they are not similar to the Lewy bodies that form in PD patients.

4. Behavioral Tests

Part of the problem with studying PD in animals is not simply the model, that is chosen, but in addition the assays used to assess changes between the healthy and diseased state. PD patients experience many motor symptoms including akinesia, bradykinesia, muscular rigidity, dystonia, resting tremors, gait abnormalities and postural instability due to progressive dopamine neuron loss and dysregulation of dopamine-modulated pathways in the basal ganglia [37, 38]. When assessing behavioral changes in rodent models, it is important to keep in mind that although the neuroanatomical components underlying motor control may be similar for humans and rodents, the manifestation of these motor deficits may be expressed differently between species.

There are various behavioral tests for rodents that are used to measure dopamine-induced motor deficits in animal models of PD. For example, there are exploratory tests such as the open field test and swim test, and then there are learned and/or innate skill tests. The latter tests include the rotarod, grid test, adjusting steps, inclined beam traversal, climbing down a pole, forelimb placing test, reaction-time test, staircase test, paw retraction test, adhesive removal and nesting behavior (for a full description of the tests see [37, 39]). These behavioral tests were largely designed to assess the innate motor skills/abilities of animals that are dopamine dependent, in order to relate the changes observed to the motor deficits seen in PD patients. However, many of these behavioral tests (with the exception of the stepping test) require the animal to learn the task first as most of these measures are complex tasks. Complex tasks can still measure innate motor skills though one does not know if the failure to perform a task is from a motor deficit or from a learning deficit. It is important to note that not all animals learn these complex tasks even prior to receiving the dopamine lesion and often are excluded from the results. In the animals that do learn the behavioral tasks one must keep in mind that the tests are reflective of akinesia and bradykinesia, and not necessarily tremor and rigidity. Although there are behavioral models that measure tremor and rigidity [39] the latter two symptoms are subtler and would probably be easier to characterize if rodents were less dependent on all four limbs for balance (for more information see Timothy Schallert's lab website: <http://homepage.psy.utexas.edu/homepage/group/SchallertLAB/>). To date, there are no behavioral models that can reproduce all of the motor deficits that are commonly seen to be in PD patients.

Another key point to consider is that the design of the paradigm influences the behavioral outcome. For example, the degree of dopamine loss, the timing and dose of the toxin injections, the time between injections and the behavioral

testing and genetic manipulations will all impact the results of the behavioral study. When comparing the MPTP and 6-OHDA lesion models, the MPTP model would seem more favorable as it produces a bilateral dopamine lesion that can be delivered using a chronic regime [40, 41], similar to the slow onset of idiopathic PD, whereas the 6-OHDA model is classically a unilateral lesion [42], although bilateral lesions have been established [43–45]. In the classic unilateral 6-OHDA model only a single injection into the medial forebrain bundle is required to induce a full dopamine lesion approximately 2 weeks after injection. This is similar to what is seen in the bilateral 6-OHDA lesion models. The bilateral lesion models may be considered more relevant to PD since both hemispheres are dopamine depleted and they can have more specificity towards behavioral impairments depending on the dose and location of the injections [46]. Although both 6-OHDA models reproduce the major behavioral deficits seen in PD, the effect of the 6-OHDA toxin does not mimic the progressive loss seen in PD. The MPTP model also has its own caveats in that the extent of neuropathology observed is dependent on the age, sex, and strain of mouse used in the study [47]. In addition, the MPTP mouse models (as with the other toxin models of PD) fail to encompass the wide assortment of motor impairments seen in PD patients [37, 48]. Perhaps the current rodent models of PD would be more predictive of what will translate into human studies if the time course of dopamine neuron degeneration could be mimicked and behavioral tests were designed to assess the more subtle symptoms of tremor and rigidity.

Beyond the paradigm chosen for a particular study, there is a concern that applies to all animal research that is often neglected when interpreting results. There are factors introduced to the everyday laboratory environment by the experimenter that can cause undue stress to the animals. For example, rodents by nature are social creatures, and follow a social dominance hierarchy. Often a dominant male will suppress his subordinate cage mates by fighting and/or guarding the food and water to establish the hierarchy. Social interactions of this nature can lead to changes in dietary intake and overall behavior, an unwanted situation when conducting a behavioral experiment. The animals can also identify with the experimenter's smell (e.g., perfumes/colognes and scents from shampoos, deodorant, laundry soaps and lotions), including that of their lab coat. By using one specific lab coat only for behavioral testing throughout the entire experiment, animals can identify with the experimenter's smell and may be less stressed by their presence. Overall, it is important that investigators consider these subtle, though potentially important, confounds to their work.

5. Physiological Concerns

Although there is a great deal of similarity between the physiology of rodents and humans, it is clear that significant differences exist. Perhaps one of the most relevant examples of this difference with regards to PD research is the distinction between how humans and rodents metabolize MPTP. Rats

and mice are relatively resistant to MPTP, whereas humans are quite sensitive to this toxin. The sensitivity of humans to MPTP became apparent in 1983 when several drug addicts unfortunately injected themselves with MPTP thinking it was synthetic heroin. These young drug addicts very quickly developed symptoms similar to PD [49]. In contrast to this, MPTP is more effective when administered with the adjuvant probenecid (which blocks the rapid clearance of MPTP and its metabolites from the kidney) in rodents in order to produce some of the pathophysiological and behavioral symptoms seen in humans [40, 41]. There are likely to be additional differences in the metabolism of environmental toxins between rodents and humans that have not yet been identified and, therefore investigators must remain cautious in interpreting the results from studies of rodent models.

Differences between the blood brain barrier in humans and rodents must also be considered in this regard. There is evidence that the neuroinflammation associated with PD may make the blood brain barrier more leaky than in a healthy individual [50]. The function of the blood brain barrier is to act as a physical and metabolic barrier between the blood and central nervous system. If this barrier becomes leaky, immune mediators of the blood may enter the brain and contribute to the neurodegenerative process. Similar to humans, the blood brain barrier also becomes leaky in rodent models of PD [50]. The brain endothelial cells from rodents do not express the same enzymes as humans, however, and therefore the influx of nutrients that nourish the brain and efflux of toxic metabolites may be different between the species [50]. The transporter differences in the blood brain barrier between species again suggest that caution is required when applying data from animal studies to humans.

6. Regulation of Gene Expression

In the past, it was thought that transcription factors were conserved in sequence and function, allowing regulation of the same target genes across species. Recent studies, however, have now shown that although transcription factors may be conserved across species, the sites which they bind are different [51]. The divergence in the cis-regulatory networks between humans and mice was demonstrated in hepatocytes [52]. When the transcription factor binding sites in human chromosome 21 are compared to the orthologous regions in mice, only one-third to a half are conserved [53]. When mouse transcription factors were placed in a mouse nuclear environment, a human-like binding signature was observed on a human-derived chromosome indicating that the human chromosomal sequence is responsible for the placement of the transcription factors [53]. Studies similar to this have not yet been done in the brain, but the existence of cis-regulatory species-specific networks suggest that we cannot assume that the regulation of gene expression will be the same between humans and the animal models used for PD research. In this regard, major differences in the expression of transcription factors were observed between human and chimpanzee brains, which most likely results in coordinated differences in the expression of downstream genes [54].

Of particular interest to PD research, differences between the transcription regulation of human and mouse tyrosine hydroxylase have already been noted [55]. This is of interest because tyrosine hydroxylase is the enzyme that catalyzes the hydroxylation of tyrosine to produce L-dopa [56], which is the rate-limiting step in the synthesis of catecholamine neurotransmitters [57].

Species differences in posttranscriptional regulation of gene expression are just as important to consider as transcriptional changes when evaluating animal models. The regulation of alternative splicing plays an essential role in the diversity of proteins produced from a single gene. To determine the extent of alternative splicing in different species, Brett and colleagues studied expressed sequence tags and determined that the extent of alternative splicing is similar among species including humans and rodents [58]. Recently, however, it was shown that humans have more regulated alternative splicing than rodents using a similar approach [59]. The different results obtained in these two studies are most likely due to the fact that the newer study used only bona fide alternative splicing events, along with a few additional differences in the methodology [59]. Although some alternative splicing events have been evolutionarily conserved, the majority of these events have not been conserved between humans and mice [60]. With regard to the most prevalent form of alternative splicing, exon skipping, it has been estimated that >11% of the events are species-specific [61]. The results from all these studies combined suggest that species-specific alternative splicing has the potential to produce large differences in phenotypic complexity. These findings suggest that we must use caution when interpreting results from studies of animal models of PD because subtle molecular changes at the level of gene expression may result in large changes in signaling pathways and behavioral and physiological responses.

In addition to splicing changes in gene expression, non-coding microRNAs (miRNAs) fine-tune gene expression by binding to RNA sequences within the 3'-untranslated region and usually downregulate gene expression by destabilizing the RNA or inhibiting translation. Many miRNAs have been evolutionarily conserved, and there are many highly conserved motifs in the 3' untranslated region of mRNAs in vertebrates, some of which most likely bind miRNAs [62]. Unfortunately, very few of the putative miRNA binding sites that have been identified through bioinformatics studies have been experimentally tested. Because of the importance of using animal models for studying diseases, further studies designed to assess the degree of evolutionary conservation of miRNA regulation of gene expression between species would be extremely helpful.

7. Conclusions

Rodents and nonhuman primates are an important resource for the study of PD, but the limitations of these models must be kept in mind when interpreting results. Nonhuman primate models are anatomically, physiologically, and behaviorally more similar to humans, but they are rarely

used because of cost and ethical concerns. Rats and mice are widely used for modeling PD, but no toxin or genetic model completely reproduces the pathophysiology seen in humans. Because it is currently thought that environmental factors and genetic susceptibility play a role in the onset and progression of PD, perhaps the most promising models are those that combine genetic models with exposure to toxins.

Because of the current limitations with PD models, some studies are best done in the clinic. An example of this type of study would be the search for noninvasive biomarkers of PD. If one is attempting to identify blood biomarkers of PD, the investigation could be done directly in humans and therefore the results obtained from the study would be directly applicable to patients.

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