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Pre-clinical screening model organism to study Parkinson disease: *Caenorhabditis elegans* and its utility

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Abstract
The people suffering from mental disorder are frequently high at 21st century due to lifestyle. Alzheimer type or Parkinson's disease is an old age disorders observed in many people in almost all countries and impose on challenge to research field for drug discovery for preventing and restoring the mental health of people. Many model such as Zebrafish, flies, anurans and nematodes (*C. elegans*) were used to study Parkinson disease but *C. elegans* are the best model for Parkinson's disease because of its special character like short lifespan, transparent and micro in size help the researcher to discover drug very easy, fast and in an economically feasible manner.

Keywords: Parkinson's disease. *C. elegans*, neurons, model

Introduction
Parkinson's disease (PD) belongs to group of conditions called motor system disorders, which are the result of the loss of dopamine-producing brain cells. The four primary symptoms of PD are tremor or trembling in hands, arms, legs, jaw and face rigidity or stiffness of the limbs and trunk, bradykinesia or slowness of movement and postural instability, impaired balance and in coordination. PD can be well studied in Zebrafish (*Daniorerio*), flies (*Drosophila melanogaster*), anurans (frogs and toads) and nematodes (*Caenorhabditis elegans*). These models cannot replace rodent and primate-based ones; as they offer convenient systems with in which to explore the relative contribution made by genetic and environmental factors to PD pathology. In addition, they offer an economic and rapid alternative for testing compounds which target a PD. Most importantly, the combined use of these models allow to uncover the basic mechanisms underlying PD pathogenesis (Pienaar *et al.*, 2010) [12]. These models will fit into the new concept of drug discovery 3 Rs (Reduction, Refinement and Replacement) (Clark, MJ. (2018)) [13].

Current PD models used for understanding PD therapies is through of disease mechanisms and improving therapeutic outcomes by rodent and primate models which have been generated by administering neurotoxic substances or via genetic manipulation. In these models stereotaxic or systemic delivery of neurotoxins such as 6-hydroxydopamine (6-OHDA), rotenone, paraquat or 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), this is converted to the potent neurotoxin 1-methyl-4-phenylpyridinium. These toxins all selectively destroy nigrostriatal DA neurons, producing a syndrome that resembles idiopathic PD in animals (Burns *et al*., 1983) [14]. Intense efforts have been made towards developing improved PD models for better understand the etiology and pathogenesis of PD, and to identify new drug targets. However, neither toxin induced nor transgenic animal models of PD, perfectly recapitulates all human symptoms. Toxin models have advantage for studying DA deficiency and the effects of DA replacement therapy. Transgenic approaches include the over expression, knock-out, knock-in and knock-down of PD genes (Lewis *et al*., 2008) [15]. For instance, since Parkinsonism also characterizes individuals with fronto-temporal dementia, expression of mutant form microtubule-associated protein tau, has been shown to cause severe early-onset Parkinsonism in mice (Ittner *et al.*, 2008) [16]. However, in the context of the current emphasis on high throughput screenings to identify genetic interactions or pharmacological therapies, murine or non-human primate models often prove costly and time-consuming (Faust *et al*., 2009) [17], hence models using fish, worms, flies and anurans have become attractive alternatives and have made a significant contribution towards better understanding disease mechanisms and uncovering novel therapeutic strategies.

The Development of pre-clinical model for parkinsons screening

The development of pre-clinical models amenable to live animal bioactive compound screening is an attractive approach to discovering effective pharmacological therapies for disorders caused by misfolded and aggregation-prone proteins. In general, however, live animal drug screening is labor and resource intensive, and has been hampered by the lack of robust assay designs and high throughput work-flows. Based on their small size, tissue transparency and ease for cultivation, the use of *C. elegans* should obviate many of the technical impediments associated with live animal drug screening. Moreover, their genetic tractability and accomplished record for providing insights into the molecular and cellular basis of human disease should make *C. elegans* an ideal model system for *in vivo* drug discovery campaigns.

This model provides powerful validation for advancement in preclinical drug discovery campaigns by screening live *C. elegans* modeling a1-antitrypsin deficiency and other complex disease phenotypes on high-content imaging platforms.

The nematode *Caenorhabditis elegans* offers several advantages as a tool for studying PD, including its relatively short lifespan, lasting 20 days on average, low costs in growing and maintaining large colonies. Genetic, genomic and chemical mutant screens are more easily performed *in C. elegans* than in most other experimental species. Specifically, RNA interference (RNAi) is particularly easy in nematodes, since worms that can be grown on agar plates where they feed on bacteria that express small interfering RNA (siRNA) to down regulate expression of distinct target genes (knock-down). Compound screening using *C. elegans* is also less time-consuming, due to fast reproduction and a high progeny number (Schmidt *et al.,* 2007) [18]. The basic cell biology and biochemistry of nematodes overlap with mammals, including similar ion channels, neurotransmitters (e.g. DA, serotonin, acetylcholine, GABA, etc.), vesicular transporters, receptors and synaptic components. The wiring diagram of the nematode nervous system consists of a defined set of 302 neurons, of which 8 are dopaminergic. The discovery that application of exogenous DA inhibits locomotion and egg laying behavior in this species led to developing *C. elegans* animal models for PD. Mutant worms that lack TH, and are therefore unable to synthesize DA, show a deficit in the ‘‘basal slowing response’’, a food-foraging behavior that depends on specific dopaminergic neurons termed ADE, PDE and CEP neurons. Laser-assisted targeting of the dopaminergic system disrupted area-restricted searching behaviors employed by nematodes in locating food, thereby demonstrating that this behavior depends on dopaminergic signaling (Schmidt *et al*., 2007) [18].

Transgenic worms that over express wild-type and mutant forms of human SNCA (A30P and A53T) in DA neurons caused an accumulation of a SYN in these neurons. Moreover, worms expressing mutant forms of human SNCA failed to modulate the locomotor rate in response to the availability of food, a function normally attributed to dopaminergic neurons. This behavioral abnormality was accompanied by a reduction in neuronal DA levels, which was rescued by administering DA (Kuwahara *et al.,* 2006) [19]. In addition, worms expressing human SNCA under control of the promoter for DAT displayed age- and dose-dependent dopaminergic neurodegeneration (Cao *et al.,* 2005; Hamamichi *et al.,* 2008) [20, 21]. Nematode DA neurons exposed to 6-OHDA undergo apoptosis within 6 h of toxin exposure (Nass *et al.,* 2002) [22], with complete neuronal destruction detected after 72 hours and larval arrest was also seen following rotenone administered to *C. elegans* (Schmidt *et al.,* 2007) [18], while treatment with MPTP evoked decreased motility and degenerated DA neurons (Braungart *et al.,* 2004) [23]. All these Parkinsonian toxins are used in rodent and non-human primate models, and, therefore, the results, such as discussed above, obtained in models other than such traditional animal models of PD offer encouragement (Pienaar *et al.,* 2010) [12].

C. elegans has been extensively studied and characterized by various researchers, extending into many realms, including the generation of a complete cell lineage map and an extensively studied genome. The *C. elegans* nervous system is well-characterized and a complete wiring diagram is available. The *C. elegans* nervous system contains only 302 neurons of 118 subtypes, 6393 chemical synapses, 890 electrical junctions, and 1410 neuro muscular junctions. The presence of strains expressing fluorescent proteins in specific neuronal subtypes allows for the live worm to be observed. Additionally, the functions of many of these neurons have been determined by laser ablation and drug exposure studies allowing behavioral studies to reveal alterations in neuronal networks. *C. elegans* can be cultivated on solid support or in liquid medium and can be exposed acutely or chronically to toxicants by injection, feeding, or soaking. Automated imaging methods for assessing absorbance, fluorescence, movement, and obtaining morphometric measurements were developed beginning in the late 1980s. Recently, expression of fluorescent proteins combined with imaging platforms have been used successfully for large-scale promoter expression analyses and drug screening purposes. Additionally, the application of microfluidics technology to biological assays in *C. elegans* allows the use of three-dimensional imaging and high complexity assays (Rohde *et al*., 2007) [24].

The dopaminergic system in *C. elegans* is particularly simple to visualize using the DAT-1: GFP strain. Containing only eight dopaminergic neurons in the hermaphrodite (four CEPs that extend processes to the tip of the nose, two ADEs in the head, and two PDEs in the body of the worm researchers have examined these neurons following exposure to toxicants. As degeneration of the dopaminergic system has been associated with Parkinson's disease (PD), researchers have examined alterations in this system following exposure to 1-methyl- 4-phenylpyridinium (MPP+) which induces a non-hereditary form of PD and have observed degeneration of dopaminergic neurons. Conversely, other researchers have investigated the effect of uranium (U) on the dopaminergic system to find no alterations or degeneration, a finding that correlates with data from mammalian neuronal cultures. These studies display the utility of *C. elegans* and its value as a system in which researchers can view the entire, intact nervous system in a living animal (Jiang *et al.,* 2007; Sulston., 1983; Sulston *et al.,* 1983) [25, 26, 27]. The *C elegans* model has been used to reduce the cost of screening, high speed and strong predictive value and this technology fulfills the requirement for first pass assessment of chemicals of the agents mentioned above, those that come to market will most likely represent no more than minor refinements in proven therapeutic strategies, rather than major therapeutic breakthroughs (Allard *et al.,* 2014) [28]. The quest for a major breakthrough, which is a treatment that will halt or reverse the progression of PD (approaches known respectively as neuroprotective and neuro restorative) continues to be the focus of much scientific inquiry. Nonetheless, the next generation of treatments on the horizon will likely enhance the ability of clinicians to control the

motor and non motor symptoms of PD and offer at least a modest improvement in quality of life for the people who suffer with PD.

A key feature of the disease is a diminished control over voluntary movement and progressive depletion of brain dopamine (DA) levels that stems from the large-scale loss of DA-producing neurons. Parkinson's disease (PD) was one of the first neurological disorders to have aspects of the disease modeled faithfully in non-human animal species. Despite their inherent limitations, rodent and non-human primate models of PD have helped unravel several aspects of PD pathogenesis. This paved the way for the neurotransmitter replacement therapy for PD, and a number of neuroprotective compounds that can be assessed in clinical trials. Hence the alternate model that can be used for high throuput screening is desirable at this juncture to break through the search for the new molecules to improve upon the lives of the PD patients.

Conclusion

C. elegans can be a better model to unravel the molecular level key checks can be used as a screening tool, due to its transparent body and GFP or Fluorescent labelles neurons can be created, various mutants, RNAi, gene knock outs and various behavioral parameters (Paralysis, movement, turns, food search, pharyngeal pumping and various end points can be assessed as a recovery and normalcy after treatment can be evaluated to know the restorative/ neuron protective compound or molecule.

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