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Zebrafish as a Model Organism for Parkinson's disease

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ABSTRACT

The human brain is responsible for carrying out many complex tasks that are essential for the survival, successful and healthy functioning of an individual. These functions result from the cooperation of roughly 100 billion neurons. Neurons communicate with each other by secreting neurotransmitters. Dopamine is major neurotransmitter in brain whose absence hurts motor control. Parkinson's disease (PD) is the second most common progressive neurodegenerative disease after Alzheimer's disease. It results from the slow degeneration of dopaminergic neurons in the substantia nigra. Zebrafish has been widely used as a model organism for various diseases. Zebrafish have many favorable properties as experimental animals. The embryos are small and transparent, and as adults, they are inexpensive and easy to maintain, develop rapidly, and breed in large quantities. Here, we review detailed information of zebrafish as a model organism for Parkinson's disease.

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INTRODUCTION:

Parkinson's disease (PD) was first described in 1817[1] by James Parkinson and is the second most common neurodegenerative disorder, after Alzheimer's disease. The incidence of PD increases after the age of 50 and rapidly increases after the age of 75[2]. Onset of PD is clearly age-related, and its prevalence will increase as the population ages. PD is characterized by motor and non-motor features. The most common motor symptoms are tremor, rigidity, akinesia (lack of movement), bradykinesia (slow movements), hypokinesia (reduced movement), that occur mainly due to the degeneration of dopaminergic neurons, involved in the movement coordination and located in the *substantia nigra pars compacta* (Figure 1). However, in later stages of the disease, it affects other brain regions [3,4]. The degeneration of these wider circuits in the brain is responsible for the non-motor features of the disease, such as cognitive decline, depression and, in some cases, hallucination episodes.

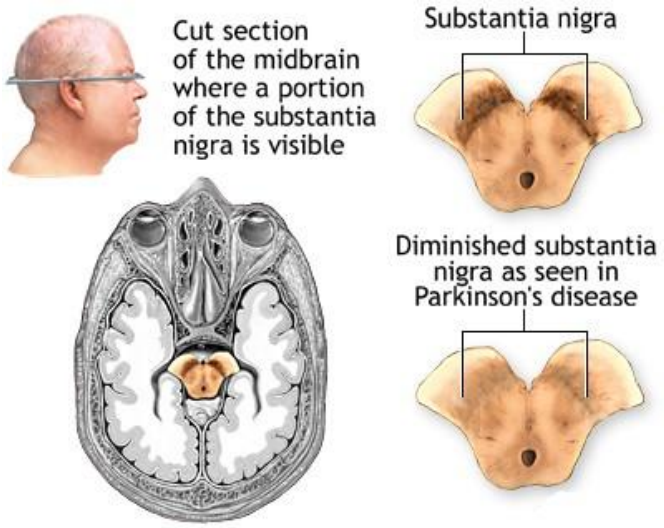

Figure 1. Spatial location of the *substantia nigra* in human brain.

In PD patients it is visually possible to detect a depletion of dopaminergic neurons in the *substantia nigra* which is responsible for the majority of the motor symptoms found in the disease.

The majority of the PD cases are sporadic and only 2% of the cases are familiar, being associated to specific gene mutations. Phenotypically, both PD forms are very similar regarding motor symptoms, suggesting that the insult responsible for the disease development and progression may be identical in both cases [5,6].

The major pathologic hallmark of PD, besides the degeneration of dopaminergic neurons, is the presence of cytoplasmic protein inclusions named Lewy Bodies (LBs) that can be found in the remaining surviving neurons. These inclusions are mainly constituted by alpha-synuclein (a-syn) which was the first protein to be associated to the disease. The role of these bodies is still unknown, however, it is believed that they may present a protective effect in the disease, by sequestering dysfunctional and toxic protein species responsible for neurodegeneration [7]. The neuropathological hallmarks of PD are loss of nigrostriatal dopaminergic neurons and presence of intracellular alpha-synuclein, parkin, and ubiquitin contained in Lewy body (LB) inclusions[8].

Etiology:

The etiology of PD is currently unknown. It is believed to be caused by interplay of genetic and environmental factors. 1-methyl-4-phenyl-1,2,3,6-tetrapyridine(MPTP) was first produced as a side-product of MPPP (a type of synthetic heroin) synthesis and identified as a cause for parkinsonism in a college student in 1976 and in several heroin addicts in 1982[9]. Pesticides have been suspected of causing PD because of their mechanism of action – causing dysfunction in the respiratory chain of mitochondria. Meta-analyses of epidemiological data suggest that a positive association exists between pesticide exposure and PD[10,11]. In addition, the pesticide rotenone is used to produce experimental parkinsonism. Somewhat surprisingly, cigarette smoking is inversely associated with the risk of PD[12], which may be due to the inhibition of MAO.

Treatment:

The treatment of PD is currently symptomatic, as no preventive, curative, or even disease-modifying treatment is available. L-DOPA, a precursor of dopamine, is the oldest and most efficacious drug for treating PD. However, long-term use of L-DOPA produces motor fluctuations and dyskinesia's and thus, treatment is usually started with other medications. These include amantadine, monoamine oxidase B (MAO-B) inhibitors, anticholinergics, and dopamine receptor, agonists. Neurosurgical options exist, such as deep brain stimulation of the subthalamic nucleus, which may be used after symptoms can no longer be managed with medication. There is an urgent need for new, disease-modifying treatment options for PD.

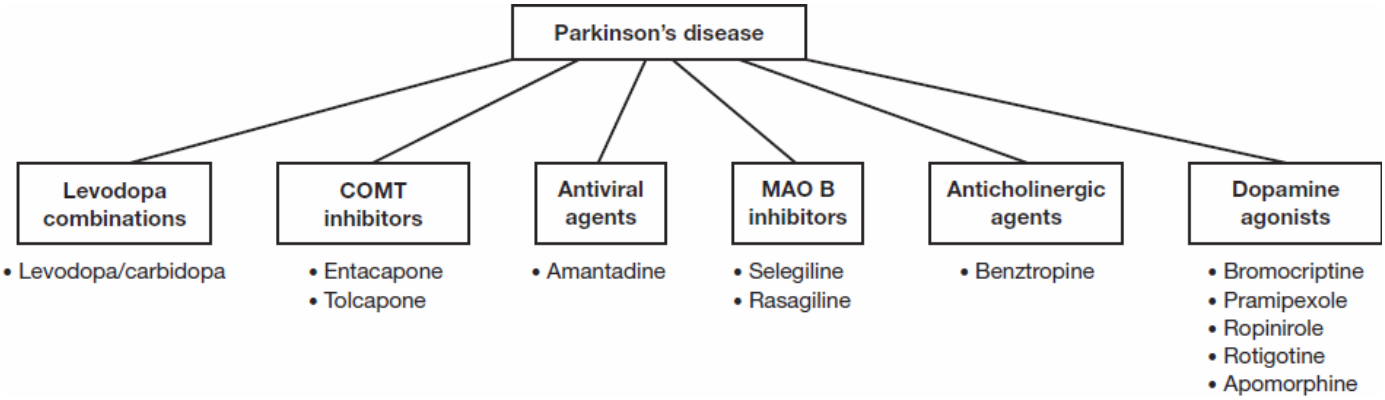

Figure 2. Classification of Antiparkinson's drug.

Introduction to zebrafish:

Zebrafish are a small fish usually less than 5 cm in length with stripes on the body. Geographically we can find them from Pakistan to Myanmar and from Nepal to Karnataka in India. They live in slow moving water like ponds, lakes, rice paddies *etc*. They are commonly kept as aquarium fish, as they are easy to maintain. Zebrafish were first introduced for biomedical research purposes by George Streisinger in 1981. Streisinger chose zebrafish, among other species, due to their ideal combination of properties. Zebrafish have a tendency to live in shoals. They are active during the day and have a rest at night. They are omnivorous. Naturally, it feeds with zooplankton and insects[13].

Zebrafish as a model animal:

Zebrafish as a model animal progressed to be used during the 1960s and when the zebrafish genome started to be sequenced, their use rapidly increased (post-1996)[13]. Because their genetic sequence is quite similar to humans they became popular as a model animal for human diseases[14]. Now they are used mostly in molecular biology, developmental biology, neurobiology and genetics research[13].

Advantages of zebrafish as an animal model are its small size, short generation time and easy and cheap maintenance [15]. Another advantage of zebrafish larvae is that larvae are transparent so it is possible to observe the development of organs and tissues[16]. Their development is fast. Larvae one week old are already able to hide from predators, catch small preys or stabilize position in moving water[17].

Fish gender identification:

Zebrafish are mature when they are 3 months old [18]. Zebrafish male and female look very similar but it is important to recognize them. Zebrafish male (Fig. 3B) are more straight and narrow shaped with darker blue stripes. They are more golden, especially on the ventral fins. They have not gotas big belly as zebrafish female. They tend to be more active than zebrafishfemale. In contrast, zebrafish female (Fig. 3A) are more pale with bluish-white stripes and have bigger white belly than male zebrafish. Also, they have more visible typical oviduct in the caudal belly region [18].

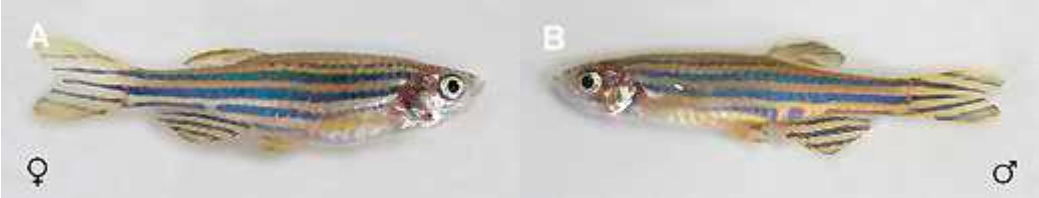

Figure 3. Zebrafish female and male: Zebrafish female (A) is characterized with bluish-white stripes and bigger belly. Zebrafish male (B) is characterized with straight and narrow shaped body with more golden fins.

Zebrafish as a model organism in PD:

The zebrafish, Danio rerio, a vertebrate model organism for genetics that has emerged in the past decade or so, is a relatively uncharted system for modeling Parkinson's disease (PD).

It has become a widely used model system for the study of development and gene function. Zebrafish are vertebrates and therefore more closely related to humans than other genetic model organisms such as *Drosophila* or *Caenorhabditis elegans*. They are relatively small fish (3–4 cm long as an adult) that can be easily managed in large numbers in specialized facilities. Zebrafish have a short generation time (3 months) and breed prodigiously (hundreds of offspring per female per week). Embryos develop externally, can be readily manipulated genetically and are transparent. Many factors suggest that the zebrafish is a powerful tool for the study of human diseases: patterning, pathfinding and connectivity in the CNS have all been deciphered and correlate with the human CNS; transparency of embryonic zebrafish facilitates analysis of single neuron activity during the execution of normal and pathological behavior[19]; touch and behavioral responses such as movement patterns can be monitored; and cardiovascular, anti-angiogenic and anti-cancer drugs elicit compatible

responses in zebrafish embryos to those in mammalian systems[20]. Zebrafish mutations phenocopy many human disorders and the genome sequence of zebrafish is near completion[21].

Induction of Parkinson's disease in zebrafish:

Some of the toxins known to induce DA cell loss in other animal models have now also been tested in adult zebrafish[22]. Four toxic substances are commonly used to produce experimental parkinsonism, they are MPTP, 6-hydroxydopamine, rotenone, paraquat.

Systemic injection of MPTP or 6-hydroxydopamine did not alter the number of DA neurons, but DA and noradrenaline concentrations in brain tissue were significantly decreased without a concomitant change of tyrosine hydroxylase (TH) or caspase 3 protein levels. The swimming velocity and total distance moved decreased after exposure to both neurotoxins. Apoptosis was not significantly increased in toxin-exposed fish. The lack of a clear decrease in the DA cell population may be due to the toxin exposure protocol; perhaps the single acute exposure (intramuscular injection) of neurotoxin, while enough to alter swimming behaviour and global brain levels of catecholamines, was an insufficient insult to result in neuronal cell loss.

Given the ease of delivering chemical compounds to zebrafish (by simply adding them to the tank water, which enables access to the CNS), the potential PD-inducing effects of MPTP, its metabolite MPP, and the pesticides including rotenone and paraquat, have been evaluated in both larval and adult zebrafish[22,23,24,25]. Motor behaviour (e.g. Swimming) can also be altered by administration of the pesticides rotenone and paraquat in both larval and adult zebrafish[23]. Adult fish were exposed to rotenone and paraquat via immersion (pesticides diluted in tank water) and exposed to MPTP and MPP+ via intraperitoneal injections. In adult zebrafish, only the highest single dose of MPTP resulted in a measurable effect on locomotor activity, and no effect was seen with rotenone or paraquat at sublethal doses.

In addition to neurotoxin-induced loss of DA neurons, it has been well established that both antipsychotics and antidepressants can have extrapyramidal side effects (EPS) leading to movement disorders in individuals who are treated with these medications. This so-called drug-induced Parkinson is usually developing within 1 month of the initiation of the offending medication in approximately 60% patients and in approximately 90% within 3 months. The likely risk factors include prior history of movement defects, age, gender, and

genetically determined differences in drug metabolism and possibly drug action. The conditions are generally reversible, once the medications are removed, suggesting that the drugs produce an interference with neuronal function rather than killing the neurons. Antipsychotics fluphenazine and haloperidol also impair locomotor activity in larval zebrafish.

Behavioral parameters in zebrafish for testing catalepsy:

Catalepsy is the major symptom of Parkinson's disease. It can be induced in zebrafish using standardized dose of haloperidol (9 μg) by giving direct exposure to fishes. During induction of catalepsy, fish will start showing aberrant swimming patterns like upside down, arrow like swimming, circular swimming, and finally state of complete catalepsy can be achieved.

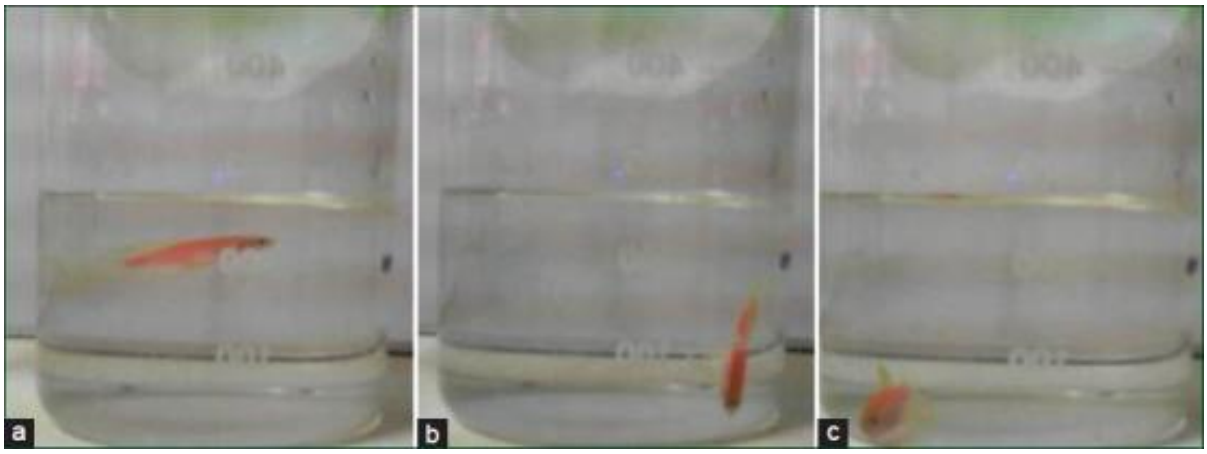

Figure 4. Induction of Catalepsy in zebrafish in haloperidol solution: (a) Fish just introduced in haloperidol solution (b) Aberrant swimming patterns shown by fish after some time (c) State of complete catalepsy

Examination tank will be filled with fresh aerated tank water. It consisted of a 5-L tank (30 × 15 × 10 cm, length × height × width) with number of vertical lines drawn on one of the faces of tank at the spacing of 5 cm and with one horizontal line which divides the water filled portion of the tank into two equal halves. Vertical lines on tank are used to calculate the speed of fish by measuring the time taken by fish to travel from first vertical line to last and horizontal line gave idea about the time spent in the upper and lower half of the tank by the fish.

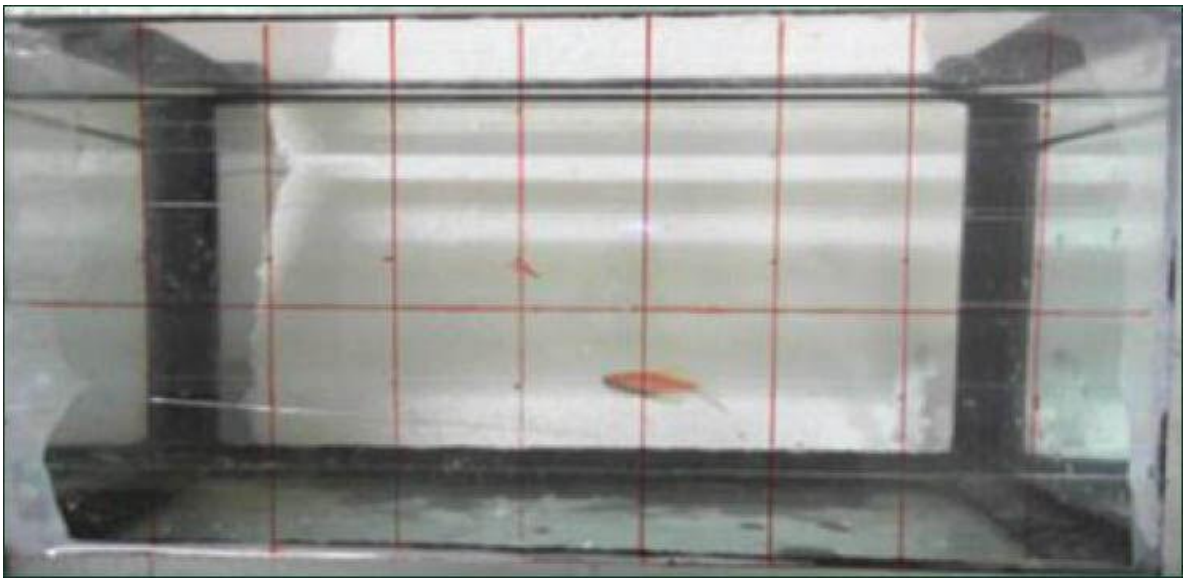

Figure 5. Examination tank used for behavioural evaluations.

Following behavioral parameters in zebrafish will be evaluated:

1. Latency to travel from one fixed point to another: In this time taken by the fish to travel from first vertical line to last was calculated. This gives an idea about the speed of fish under examination.

2. Complete cataleptic time: Time for which the fish did not move at all, i.e., the time for which fish remained completely cataleptic.

3. Time spent near the bottom of the tank: Time spent below the horizontal line drawn on tank will be measured here. This will give an idea about the anxious behavior of the fish under study.

1) Latency to travel from one point to another: Catalepsy diminishes the speed of fishes due to rigidity of muscular movements. After induction of catalepsy, rigidity of fins will be observed due to which there will be difficulty in swimming experienced by the fishes. Thus, they took longer to travel from one particular point of tank to another. Keeping this into consideration, time taken by fish to travel from first vertical line of examination tank to last line will be measured at different time interval.

2) Complete cataleptic time: Time for which the fish will be in completely immovable state will be used as index of locomotor activity in fishes.

3) Time spent near the bottom of the tank: It is a well-known fact that zebrafish are surface fish, i.e., they swim near the surface of the water. When they are transferred to a new environment (tank) they initially spend more time near the bottom of the tank and after some time they come toward surface, this is attributed towards their exploratory and most often due to their anxiety. Thus, the time spent near the bottom of the tank gives idea about the extent of anxiety of fish. It will be calculated by measuring the time spent by the fish below the horizontal line drawn on the examination tank[26].

Genetic Models of PD in Zebrafish:

PD-associated genes:

Investigation of affected families has led to the identification of some genes involved in PD pathogenesis. These include alpha-synuclein[27] and leucine-rich repeat kinase 2[28], both of which are inherited in an autosomal dominant manner and Parkin[29], DJ-1[30] and phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)[31], which are all inherited in an autosomal recessive manner, other genes-ATP13a2, synphilin-1, glucocerebrosidase, *etc.* Additionally, a very rare cause of autosomal dominant PD has been linked to UCHL1[32]. Studies of these genes have suggested that PD is caused by mitochondrial dysfunction (PINK1, Parkin), oxidative stress (DJ-1, Parkin), and protein aggregation (Parkin, alpha-synuclein, UCHL1).

Studying the development and Regeneration of da neurons in Zebrafish:

Most of the PD symptoms are caused by the degeneration of DA neurons. Replenishment of brain DA with the L-dopa or DA agonists remains the pre-dominant symptomatic treatment for PD. Transplantation of DA neurons into the patient's brain could potentially be a cure for PD. However, two major obstacles severely hinder its use: First, there is a limited availability of pure DA neurons to serve as a source for transplantation. Second, the survivability of transplanted DA neurons is poor. In order to overcome these obstacles, a clear understanding of the mechanisms underlying the development and connectivity of DA neurons is important. Moreover, an ideal treatment for PD would be to somehow stimulate the endogenous adult neural stem cells to re initiate the developmental program toward DA neurons.

CONCLUSION

Zebrafish may become very effective tool for high throughput screening for various diseases. They can be used with ease of handling, less cost and effectiveness for initial screening of drugs before testing them in rodent models. Thus, it saves large number of rodents and also it satisfies 3R's (Replacement, Reduction and Refinement) of pharmacological testing in animals.

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