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Modelling toxicity induced Neurological disorders in Zebrafish

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Abstract

Neurological disorders have become more common and prevalent. Cellular pathology and behavioural symptoms in neurodegenerative diseases although connected are still a mystery to solve with no complete cure available yet. Central pathways in neurodegeneration involves impaired ubiquitin-proteasome machinery, autophagy and mitochondrial oxidative stress. In the case of neurodevlopmental disorders, environmental toxins and genetic factors are main causative agents. We aim to create a toxicity induced zebrafish model of neurological disease focussing on cognition, movement and hyperactivity disorders. Zebra fish embryos at 48 hr post fertilization were treated with different doses of lead, cholesterol and acetyl choline and by 7 days post fertilization pectoral fin movement, swimming behaviour and touch response were compromised in parallel with apoptosis identified in the brain by acridine orange fluorescent staining. A marked window is observed, therefore promising for a drug screening platform. Further characterization of pathology associated protein expression and specific behavioural studies could render this as a simple promising toxic model for preclinical drug screening.

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Introduction

Current world wide estimates of people with the two most common neurodegenerative disease is around 18 million for Alzhiemer's disease (20) and 6.3 million for Parkinson's disease (7); prevalence of autism is as low as 6% in USA and up to 20 % in India (8). Aggregation of misfolded proteins is a common pathology shared by all neurodegenerative diseases, although nature of aggregates, and

symptoms vary(5). It is inevitable that all neurodegenerative disease could share a common link in the protein aggregation pathway and therefore in the progress of the disease. Mutations that compromise autophagy include presenilin 1 (Alzheimer Disease), huntingtin (Huntingtons Disease), α -synuclein, parkin, LRRK2, PINK1 (Parkinsons Disease), dyenin, ESCRT-III (Amyotrophic lateral sclerosis) and laforin (Lafora disease) (17). Furthermore reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important modulators of core cellular functions such as apoptosis, ion transport, and calcium mobilization leading to altered excitotoxicity and apoptosis the two key causes of neuronal death (6). It has been also shown, that induction of ubiquitin-proteasome machinery could alleviate the deposition of toxic aggregates (1). We therefore choose to induce alterations in these pathways to model neurodegenerative disorders and further to model neurodevelopmental disorders we used compounds that compromise brain development. Lead a proven neurotoxin in the developing brain of rats causes oxidative stress and apoptosis(15), cholesterol metabolism is associated with Alzheimers disease (11) and further membrane cholesterol has shown to upregulate glutamatergic receptors(18). Acetyl choline has also proven to cause glutamate – induced neurotoxicity in cultured hippocampus neurons(12). Zebrafish is a versatile model to understand developmental toxicity and becoming increasing popular(13,19,21). Creating a neuropathological state in zebrafish supported by compromised behaviour on exposure to proven chemical neurotoxins would establish a simple yet robust model. Although genetic models are outstanding as they are very specific, they are laborious and time consumptive. A toxic model validated at both cellular and behaviour levels would nevertheless suffice to screen for Hiits that could be translated to mammalian models.

Materials and Methods

Zebra fish were procured from local aquarium specialist and acclimatized for a month. For egg collection male and female zebrafish were maintained in isolated tanks for a week prior to mating, and eggs were collected in the morning after the fish were put together. The eggs were immediately transferred to sterile aquarium water. To expose for induction of toxicity Groups of 6 fry, 48hpf, were transferred to 100ml of water each containing 0.1/ml Lead, 0.1ug/ml Acetylcholine and 1ug/ml of cholesterol reconstituted with 0.01% of DMSO. Fry were maintained at 28°C, and light and dark cycle of 14/10 hours. Screening was periodically done every 24 hours upto 7 dpf.

Assay

To count the number of pectoral fin movement fry were gently transferred to a cavity slide under 4X bright field microscope and the number of pectoral fin movement were counted for every minute Neuronal apoptosis was quantified by staining fry with 0.5 ug of acridine orange and corrected total cell fluorescent intensity was quantified by imageJ software available online. Distance travelled by the fry within 10 seconds after touching the tail was measured as a response to touch. Nature of swimming was observed such as normal, slow or haphazard

Results

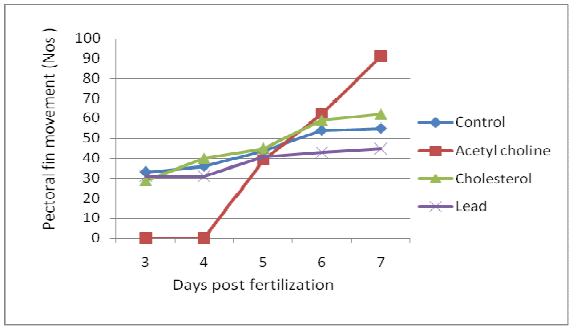

Fig 1: Graph showing number of pectoral fin movements per minute from day 3 to day 7 of 48 hpf treated embryo.

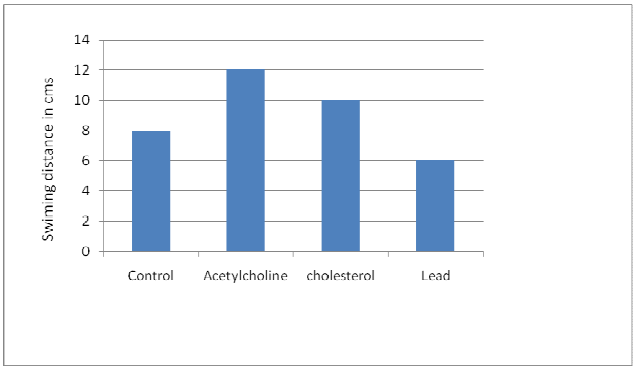

Fig 2. Swimming distance measured in centimetres, at 7 days post fertilization fish in response to touch

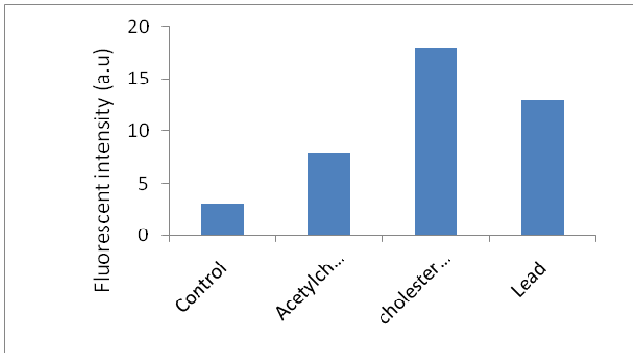

Fig 3. Fluorescent intensity of the head portion of acridine orange stained 7 dpf fish.

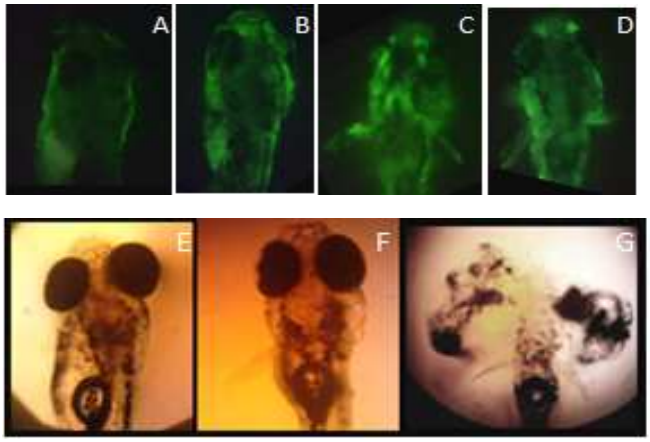

Fig 4. (A-E) 10 X Fluorescent microscopy of acridine orange stained brain of 7 dpf fish Control (A), acetycholine treated (B), cholesterol treated (C) and lead acetate treated (D). (E-F)10X Bright field microscopy of control (E) and cholesterol treated fish at 1 minute on glass slide (F) and disintegrating head of the same cholesterol treated fish at 8 minutes (G).

Discussion

The hallmarks of neurodegenerative diseases is degenerative neuronal cells leading to impaired cognitive and movement disorders. Lead treatment proves to affect cognition as in decreased swimming distance in response to touch, and impaired movement as in decreased pectoral fin movement although swimming behaviour was normal; apoptosis of brain cells is evident. In the case of acetylcholine and cholesterol treatment hyperactive or uncontrollable movement is evident as in faster pectoral fin movement and increased swimming distance in response to touch, moreover swimming motion of these fry were faster and haphazard compared to control fish. Further poor development of brain and skeletal architecture is evident in cholesterol treatment as observed in disintegration of the fry head on glass slide. Hence lead can be used to model both cognitive and movement disorders as observed in most neurodegenerative diseases, while cholesterol and acetylcholine can be used to model neurodevelopmental disease as in attention-deficit/hyperactivity disorder (ADHD). It has also been hypothesized that hyperactive glutamatergic system is connect to ADHD and Amyotrophic lateral sclerosis (10), therefore modelling with both cholesterol and lead without doubt offers more accurate replication of molecular pathogenesis in such cases. The hyperactivity is strongly pronounced enough unlike in doubtable mild compromise of cognitive functions (18). Chemical neurotoxicity is promising since it can be used very specifically to identify developmental toxicity (2,3). In the case of Parkinson's disease 6-hydroxydopamine, MPTP, rotenone and paraquat have been employed based on the investigational view(9), similarly toxic modelling hold great promise in the case of neurodevlopmental modelling since neural development and patterning can strongly be linked with the human system (19).

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