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Research Report

Neuroprotection of MPTP-induced toxicity in zebrafish dopaminergic neurons

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Abstract

Parkinson's disease is characterized by a severe loss of dopaminergic neurons resulting in a range of motor deficits. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to cause a similar loss of dopaminergic neurons in the human midbrain with corresponding Parkinsonian symptoms. Several animal species have also shown sensitivity to MPTP, including primates, mice, goldfish, and, most recently, zebrafish. This study demonstrates that the effect of MPTP on dopaminergic neurons in zebrafish larvae is mediated by the same pathways that have been demonstrated in mammalian species. MPTP-induced neurodegeneration was prevented by co-incubation with either the monoamine oxidase-B (MAO-B) inhibitor L-deprenyl or the dopamine transporter (DAT) inhibitor nomifensine. Furthermore, targeted inactivation of the *DAT* gene by antisense morpholinos also protected neurons from MPTP damage. Thus, the mechanism for MPTP-induced dopaminergic neuron toxicity in mammals is conserved in zebrafish larvae. Effects on swimming behavior and touch response that result from MPTP damage are partially ameliorated by both L-deprenyl and *DAT* knockdown.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Parkinson's

Keywords: Zebrafish; MPTP; Parkinson's disease; Neurodegeneration; Dopamine transporter

1. Introduction

Parkinson's disease is a progressive neurodegenerative disorder affecting over one million people in the United States. Symptoms include uncontrolled tremors, rigidity, slow movement, and impaired balance (Parkinson's disease foundation, http://www.pdf.org/AboutPD/symptoms.cfm). Morphologically, Parkinson's disease is characterized by a loss of the pigmented dopaminergic neurons located in the substantia nigra, resulting in depletion of the neurotransmitter dopamine. Other groups of neurons, such as the noradrenergic neurons of the locus coeruleus, can also be affected. Formation of Lewy inclusion bodies in the substantia nigra is another hallmark of Parkinson's disease (reviewed in [46]).

While the cause of Parkinson's disease remains elusive, genetic studies of Parkinson's patients have provided some clues. Rare cases of familial Parkinson's disease have been linked to mutations in several proteins, including α-Synuclein, Parkin, and Ubiquitin dehydrogenase (reviewed in [11,22]). α-Synuclein is a component of Lewy bodies, suggesting that this protein may play a role in idiopathic Parkinson's disease. The involvement of Parkin, which contains a ubiquitin ligase domain, along with Ubiquitin dehydrogenase, suggests that abnormal protein degradation may play a role in the pathology of Parkinson's disease [18].

Other studies have led to speculation that environmental toxins may contribute to idiopathic Parkinson's disease. For example, environmental neurotoxins such as rotenone have been shown to cause Parkinson's-like

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symptoms in a rat model [2]. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to cause a syndrome in humans that resembles Parkinson's disease. MPTP causes selective degeneration of dopaminergic neurons in the substantia nigra, and patients exhibit symptoms including tremors, immobility, and a shuffling gait [21]. MPTP is metabolized to 1-methyl-4-phenylpyridinium (MPP^+) in glial cells in the brain. After release from the glia, MPP^+ is transported into dopaminergic neurons via the dopamine transporter (DAT) [30]. MPP^+ accumulates in the mitochondria where it is believed to cause cell death by disrupting respiratory enzymes and causing oxidative damage [5,33].

MPTP-induced Parkinsonism has become well accepted as a model for Parkinson's disease in mammals ranging from mice to primates [3,13,17,26,38]. For example, the two most common drugs used to treat Parkinson's disease, levodopa and L-deprenyl (also known as selegiline), both ameliorate the symptoms of MPTP-induced Parkinsonism.

The zebrafish has been widely used for studies of developmental biology because the embryos are transparent, enabling the visualization of developing organs and tissues. Recently, the advantages of zebrafish have been exploited to develop models of human disease (reviewed in [20,42]). The catecholaminergic system in adult zebrafish has been well described by immunohistochemistry and retrograde tracer experiments [23–25,40]. In contrast to mammals, dopaminergic neurons in the zebrafish are found in the diencephalon and telencephalon, but not in the midbrain, where the dopaminergic neuron-rich substantia nigra is located in the mammalian brain. However, experiments utilizing a retrograde tracer coupled with tyrosine hydroxylase immunoreactivity suggested that a group of dopaminergic neurons in the diencephalon projects to the striatum in the zebrafish brain and thus may represent the equivalent of the substantia nigra neurons [40].

The embryonic zebrafish brain contains several clusters of dopaminergic, noradrenergic, and adrenergic neurons in locations similar to those found in the adult brain [14,15,27,41]. By 3 days post-fertilization (dpf), dopaminergic neurons are observed in the ventral diencephalon, the pretectum, the locus coeruleus, the olfactory bulb, and the retina. Most clusters of neurons present in the adult zebrafish brain are represented by 5 dpf [41].

The neurotoxic effects of MPTP are known to vary by species [26]. Primate brains exhibit the highest sensitivity to MPTP, while rodent brains are more variable, with the rat brain being the least sensitive to MPTP (reviewed in [9]). Evidence that MPTP has a destructive impact upon dopaminergic neurons in teleost fish has been demonstrated. MPTP damaged dopaminergic neurons in adult goldfish [36], and L-deprenyl exhibited a protective effect [12]. A recent paper showed that MPTP treatment resulted in lower levels of dopamine in adult zebrafish brains with corresponding behavioral abnormalities; however, only minimal damage to dopaminergic neurons was observed [1]. More recent studies examined the sensitivity of zebrafish neurons to several environmental toxins, including MPTP, rotenone, and paraquat in zebrafish embryos, larvae, and adults [6,19]. Only MPTP specifically affected dopaminergic neurons in zebrafish embryos and larvae.

In this study, we provide evidence that MPTP functions through conserved pathways in the zebrafish by demonstrating that MPTP-induced neurotoxicity can be prevented by treating embryos and larvae with the neuroprotective molecule L-deprenyl, by targeted inactivation of DAT using antisense morpholinos, and by a specific DAT inhibitor. L-deprenyl and DAT knockdown also appear to improve locomotor defects caused by MPTP.

2. Materials and methods

2.1. Zebrafish

Wild-type zebrafish of the Tübingen strain were maintained at 27 °C in a recirculating aquaculture system equipped with carbon filtration, ultraviolet light sterilizers, and biofiltration (Aquatic Habitats). All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Georgia State University. Tricaine was used to anesthetize animals when necessary.

2.2. MPTP protocol

Fertilized eggs obtained from mating pairs of adult zebrafish were cultured in Holtfreter's solution (60 mM NaCl, 2.4 mM sodium bicarbonate, 0.8 mM calcium chloride, 0.67 mM potassium chloride) containing streptomycin and penicillin. All experiments were performed in 24-well plates with 4 embryos in each well. Phenylthiourea (PTU, Sigma-Aldrich) was added for a final concentration of 0.003% to prevent pigmentation of embryos (up to 3 dpf) and larvae (>3 dpf). Previous studies have shown that PTU does not affect the expression of the *TH* or *DAT* genes in the developing brain [15].

MPTP was purchased in 10 mg quantities in glass bottles with rubber stoppers (Sigma-Aldrich). Stock solutions of MPTP (10 mg/mL) were made by adding water directly to the bottle with a needle and syringe. All manipulations with MPTP were performed under a chemical hood. Safety precautions included the use of protective clothing, gloves, goggles, masks, and decontamination of all surfaces and solutions with 1% bleach at the end of each experiment [37].

MPTP was diluted in Holtfreter's solution to achieve final concentrations of 5–20 μg/mL. The arrayed embryos were dosed with MPTP at 24 h post-fertilization (hpf) and placed in an incubator maintained at 28 °C. Three days later, larvae were rinsed extensively and fixed in 4% paraformaldehyde.