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Neurotrophin-3 and the degenerating brain: Lessons from the fish tank

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ABSTRACT

Introduction: Ageing has been posed as one of the highest risk factors for neurodegeneration in the population, which results in cognitive and motor decline, such as Parkinson's Disease (PD). It has been reported that the prevalence of PD in the population of more than 60 years old increased exponentially, with more than 50% of PD cases being undiagnosed. Neurotrophin-3 (NT3), a neuroprotective growth factor, supports specific neuronal populations' development, maintenance, and survival. Evidence suggests that neurotrophin levels, including NT3, fluctuate with age, potentially contributing to neuronal vulnerability. This study aimed to assess the effects of NT3 levels in MPTP-induced neurodegeneration in the zebrafish animal model. **Materials and Methods:** To evaluate neurodegeneration, PD neurodegenerative changes were induced by injecting 100µg/g of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intraperitoneally in zebrafish to induce neurodegenerative changes via mitochondrial dysfunction within 24 hours that mimics the pathogenesis of age-related PD. The fish were assessed at 24-hour, 72-hour and 96-hour post-injection. Locomotor assessment was conducted to confirm the clinical features of PD in the zebrafish model. Immunohistochemical (IHC) analysis of dopaminergic neuron degeneration was conducted to confirm neurodegenerative changes induced by MPTP. The ntf3 gene expression was analysed using quantitative polymerase chain reaction (qPCR), protein expression for NT3 and oxidative stress markers were assessed using Enzyme-Linked Immunosorbent Assay (ELISA), and brain sections were analysed based on NT3-targeted IHC analysis. **Results:** In the PD model, there was a significant reduction in mean speed (3.95 ± 0.24; $p<0.001$) and distance (1175.32 ± 72.64; $p<0.001$) in the PD model group compared to the vehicle. The dopaminergic neuron analysis showed a significant reduction in positive cells in the area of the ventral thalamus and posterior tuberculum, further confirming that neurodegenerative changes have taken place following MPTP injection. ntf3 gene expression was significantly downregulated (20-fold) following MPTP injection, with the lowest expression noted in the 72-hour post-injection group compared to vehicle (0.05 ± 0.004; $p=0.005$). The pattern of NT3 follows the ntf3 trend with the deepest pit of drop in the protein level at 72-hour of assessment following MPTP injection, where there was an 8% drop (ELISA: 3295.32 ± 186.11; $p=0.022$) with increment of caspase-3 (CASP3) level of 4.2 times higher than the vehicle group (302.40 ± 11.00; $p<0.05$). Immunohistochemistry analysis showed there was a global reduction of NT3 staining in all brain sections, not specific to PD-sensitive areas. The intensity of NT3 chromogenic stains was gravely reduced from diffuse deep intensity (+++) in the vehicle group to mild staining intensity (+) in the PD model brain. These findings reflect a marked depletion of neurotrophic support in the events of neurodegeneration. **Conclusion:** NT3 is actively expressed in the adult zebrafish brain, with significant reductions observed in a neurodegenerative brain. These findings suggest a potential role for NT3 in maintaining neuronal integrity during oxidative stress and raise the possibility of NT3-based strategies to mitigate neurodegeneration.

Keywords: Neurodegeneration, neurotrophin-3, Parkinson's Disease, ageing