1. ABSTRACT

Amelioration of 6-OHDA and MPTP induced Parkinsonism using nanoparticles of H₃-antagonist and c-Jun N-terminal kinase (JNK) inhibitor

Introduction: Parkinson’s disease (PD) is the second most common progressive neurodegenerative disorder after Alzheimer’s disease (AD) with a prevalence of 0.5-1% among people older than 65 years of age. Although the etiology of idiopathic Parkinson’s disease (PD) is unknown, this neurodegenerative disease is characterized by the loss of dopamine (DA) producing neurons in the ventral midbrain with cell bodies in the substantia nigra pars compacta (SNpc).

Objective: Recent reports suggest that the highest density of Histamine H₃-receptors is found in basal ganglia and H₃-antagonists can increase the turnover of dopamine in basal ganglia. Few other reports suggest that, c-Jun N-terminal kinase (JNK) pathway plays an important role in stress mediated neurotoxicity and inflammation; while blockade of JNK by specific inhibitors may prevent or effectively slow-down the progression of PD. The design of our hypothesis includes the development of H₃ antagonist Conessine, and JNK-3 inhibitor 1,9-Pyrazoloanthrone (1,9-P) loaded liposomal drug delivery system and the evaluation of its neuroprotective efficiency in-silico, in-vitro and in vivo.

Methodology: Followed by in silico and in vitro screening the H3 antagonist conessine and JNK-3 inhibitor 1,9-P were selected for preparation of liposomes to cross BBB effectively. The extent of neuroprotective effect of formulation were evaluated by molecular expression of anti-apoptotic and apoptotic proteins like BCL-2/BCL-XL, Caspase-3, JNK, p-38, cytochrome-c and Mitochondrial complex-I activity in SH-SY5Y neuroblastoma cell line. The 6-OHDA and MPTP induced Parkinson models were used for study and accordingly groups were made. Various parameters like level of brain striatal dopamine, antioxidant potential, and total protein content were evaluated in brain homogenate. The histopathology and iron degeneration in brain and various behavioral parameters were also estimated using appropriate methods.

Results: The preliminary in silico and enzyme kinetic study showed good potency of Conessine and 1,9-P over H3 receptor and JNK-3 respectively. Developed liposomes were characterized and found to be suiting the
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requirements for brain drug delivery. The AO/EB and Hoechst 33342 staining assay showed effectively reduction in apoptosis in treatment groups. The in vitro study showed significant reduction in apoptotic proteins like Caspase-3, JNK-3, p38, cytochrome-c, Bax and increased Bcl-2 an ant-apoptotic protein level. The biodistribution studies showed effective uptake of liposomes in brain than plain drug solution and better pharmacokinetic activity. The in vivo evaluation and striatal dopamine estimation further justify the neuroprotective dopaminergic system restoring activity of selected molecules. The histopathology and ion degeneration studies showed protection of dopaminergic neurons in treatment group from neurotoxic chemicals.

Conclusion: The present study tried to identify a non-dopaminergic pathway for the treatment of Parkinson’s disease by altering the causative molecular pathway with minimal side effects. The developed liposomes showed promising neuroprotective effect both in vitro and in vivo against 6-OHDA and MPTP induced Parkinsonism. The important outcome of study is combination therapy of selected drugs shows better and effective action than single drug treatment. The current drug therapy can lay a new path to treat the Parkinson’s disease by targeting alternative pathway than dopaminergic system.

Keywords: Conessine, 1,9-Pyrazoloanthrone, Parkinson's Disease, SH-SY5Y neuroblastoma cells, Histamine H3-receptor antagonist, JNK-3 inhibitor, Dopamine, Liposome, Basal Ganglia.