Summary of Work done

Role of novel and potential small molecules on central nervous system functions

Introduction

Central nervous system (CNS) disorders account for 12% of deaths worldwide, and those who survive are reported to have a poor quality of life. The impairment of neurological functions in CNS disorders is the result of compromised intrinsic neuroprotective, neurotrophic and neurogenic equilibrium, leading to severe damage to the structural and functional integrity of the brain. Restoring CNS homeostasis after an injury or a disease is a major challenge. Neurotrophins or neurotrophic factors, like Brain-Derived Neurotrophic Factor (BDNF) and Glia-Derived Neurotrophic Factor (GDNF), have shown promising outcomes in these restoration processes. Though the approach of exogenously administering neurotrophins seems to be a potent therapeutic alternative in treating neurological disorders, they have so far failed to cross preclinical or clinical stages because of limitations owing to its proteinaceous nature, stability in the system (bioavailability), blood-brain barrier permeability and non-selectivity. Hence, there is a dearth of therapeutic interventions that can slow down neurodegeneration and help in the repair and regeneration of neurons in the affected brain circuitry. These suboptimal pharmacological properties of neurotrophins can be overcome by using small molecules which mimic neurotrophins or which can augment the expression of neurotrophins. This has galvanized research into new compounds, including natural products, that mimic neurotrophic factors or act as neuromodulators to accelerate neuritogenesis and result in neuroprotection.

Based on all these observations, in the present work, we evaluated the *in vitro* and *in vivo* neuroactive properties of few compounds which are lichen-derived and few synthetic ones like - Fellutamide B fragments and HDAC inhibitor.

Statement of problem

Neurotrophic small molecules have been shown to ameliorate the pathophysiology of neurodegenerative and psychiatric disorders in preclinical rodent models. Nevertheless, there is a dearth of therapeutics available in clinics to promote neuroprotection, neurite regeneration and neurogenesis. Small molecules from natural resources and synthetic approach have shown potential in reversing the impaired neurotrophic milieu. Hence we

followed our interest to assess the neuroactive potential of small molecules from natural resources (Lichen derived compounds) and synthetic compounds (Fellutamide B fragments) in *in vitro* neurotrophic and neurogenic assays. In addition, the promising compounds were taken to the next level, i.e. pre-clinical studies, for their *in vivo* neuroactive therapeutic potential in chronic stress induced neuropsychiatric models in zebrafish.

Objectives of the study

1. Role of Lichen derived compound on central nervous system functions
2. Role of Fellutamide B fragments on central nervous system functions.
3. Role of novel HDAC inhibitors on central nervous system functions

Methodologies Used and Sample Results

We considered twelve lichen-derived metabolites as possible pharmacological agents for diverse neurodegenerative and neuropsychiatric disorders. The in vitro neurotrophic activity of these agents or compounds was assessed first in in vitro assays using Neuro2A cells. We observed that out of twelve only four lichen compounds (atranorin, perlatolic acid, physodic acid and usnic acid) exhibited potent neurotrophic activity and their neurotrophic potential was similar to that of a known neurotrophin NGF. In addition, we found that one of the four, perlatolic acid, exhibited more neurotrophicity than the well-studied neuroactive natural compound, resveratrol. Except for Usnic acid, all other neurotrophic compounds (atranorin, physodic acid and perlatolic acid) have shown no change in the viability of cells at the optimum neurite outgrowth concentration. The active lichen compounds identified in our study elicited the upregulation of neurotrophins BDNF and NGF to a degree comparable to that of NGF positive control. We also uncovered the underlying molecular signaling involved in perlatolic acid-induced neurotrophicity, by assessing action on the MAPK-ERK and PI3K-AKT pathways. Unlike some of neurotrophic compounds, perlatolic acid did not show enhancement in the level of pERK and pAKT. These results prompted us evaluated the levels of histone modifications (acetyl H3 and acetyl H4) in perlatolic acid-treated Neuro2A cells. The results showed increase in acetyl H3 and H4 levels, compared to the vehicle treatment, following perlatolic acid treatment; similar to what was shown in cells treated with the classical HDAC inhibitor sodium butyrate (the positive control). In addition to the potential neurotrophic action, perlatolic acid also exhibited good proneurogenic activity in *ex vivo*

neurosphere cultures at 0.5 µM. The compound perlatolic acid is taken for assessing their *in vivo* antidepressant-like activity in Zebrafish chronic stress-induced depression model. The anxiolytic and antidepressant-like activity of compounds were evaluated with Novel tank test and social interaction test. Compound perlatolic acid has shown a trend in its mood elevating effect. These results and other findings warrant further exploration of the neuroactive pharmacological properties of lichen compounds in higher *in vivo* model systems such as rodents.

Fellutamide B has been reported to have anti-tuberculosis and *in vitro* cytotoxic activity. In addition, Fellutamide B has been also observed to have NGF-induced neurotrophic activity. In this direction, researchers suggested Fellutamide B scaffold and its simplified analogues to be promising in the development of neurotrophic small molecule therapeutics to treat CNS disorders. We evaluated all four fragments of Fellutamide B (A, B, C and D) for assessing their *in vitro* neurotrophic acitivity and observed that all the four compounds have good potential for the neurite outgrowth, similar to that of NGF, and the dose at which the compounds showed optimum neurotrophic effect did not have any effect on the viability of Neuro2Acells. Furthermore, all the compounds have shown potential for the pro neurogenic activity in *ex vivo* neurosphere assay and compound C also showed the neurogenic activity in zebrafish embryos. Additionally, these compounds have shown the increase in histone acetylation at the dose which was pro neurogenic.

On summarizing the results obtained, compound #A and #C were selected for taking these further to zebrafish chronic stress model, to evaluate their anxiolytic and antidepressant activities. Comp#A and #C have shown good anxiolytic and antidepressant like activity in the Novel tank test and social interaction test. In addition, the compounds have effectively induced the levels of neurtrophin (BDNF) in zebrafish brain.

Histone deacetylase inhibitors (HDACi) have emerged as fascinating epigenetic therapeutics to target cancer and have been in clinical trial for diverse CNS disorders. However, except for the HDACi SAHA (Suberoylanilide hydroxamic acid), the other classical HDACi failed to reach clinics for their side effects including toxicity, compromised potency and selectivity. Vorinostat (SAHA that reached clinics to treat cutaneous T cell lymphoma) too has cardiotoxicity and nephrotoxicity. So, we started to screen the library of small molecules based on Tubacin (HDAC6i) and SAHA scaffolds. Novel HDACi were

evaluated for PAN HDAC inhibition. Compounds with more/equal PAN HDACi were considered for the *in vitro* neurite outgrowth. Furthermore, these compounds were also screened for antidepressant activity in zebrafish chronic stress-induced depression and anxiety model. The compound (51c) shows promising anti-anxiety and antidepressant activity in Novel tank test and social interaction test. The zebrafish brain also showed the enhanced level of BDNF when treated with the compound having antidepressant activity.