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Dissertation

Title Screening For Novel Inhibitors of Alpha-Synuclein Seeded Nucleation-Dependent Aggregation and Toxicity as a Potential Therapeutic Strategy For Parkinson's Disease

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Abstract

α -Synuclein aggregation is the key pathogenic event in several important neurological disorders including Parkinson's Disease, dementia with Lewy bodies and multiple system atrophy, giving rise to a distinct group of neurodegenerative diseases known as synucleinopathies. Although the molecular basis of α -syn toxicity has not been precisely elucidated, recent studies indicate that α -syn toxicity is mediated by a nucleation-dependent aggregation process. To elucidate the structural basis of α -synuclein mediated toxicity, we developed various methods to prepare different α -synuclein species of a defined size and morphology distribution, and we investigated their toxicity in different human dopaminergic cell lines. We observed that crude α -synuclein oligomers preparations, containing both monomeric and heterogeneous mixtures of α -synuclein oligomers, were the most toxic species. The toxicity of α -synuclein aggregates was directly linked to the presence of the monomeric α -synuclein, and strongly dependent on its ability in seeded nucleation-dependent aggregation converting into amyloid fibrils. Therefore any effort to identify compounds that could inhibit or even reverse the aggregation process should assess the effect of the potential inhibitors on the seeded aggregation of α -synuclein, among others. We screened thirty Chinese herbal medicinal compounds for their effect on α -synuclein aggregation, seeded polymerization and toxicity by employing biophysical, biochemical and cell-culture-based techniques. Among the screened compounds, only ginsenoside Rb1, salvianolic acid B, dihydromyricetin and gallic acid were shown to be strong inhibitors of α -syn fibrillation, seeded aggregation and toxicity. Our results showed that gallic acid, ginsenoside Rb1 and salvianolic acid B inhibit α -synuclein fibrillation by binding and stabilizing the structure of the soluble, non-toxic oligomers, which are devoid of β -sheet content. In contrast, dihydromyricetin was found to be able to bind to both oligomeric and monomeric species of α -synuclein. In the case of gallic acid, the inhibition of α -synuclein fibrillation is related to the compound's hydroxyl moieties whose number and position on the phenyl ring were proven to be significant for the process of inhibition, as indicated by the structure activity relationship data obtained from fourteen structurally similar benzoic acid derivatives. Overall, the compounds identified herein may represent the starting point for designing new molecules that could be utilized as drugs for the treatment of Parkinson's Disease and related disorders.

Research Relevance and Potential Impact

The accumulation of aggregated form of α -synuclein (α -syn) protein in the brain has been found to be the main characteristic of Parkinson's disease and related disorders. Recent studies indicate that α -syn toxicity is mediated by a nucleation-dependent aggregation process. Therefore any effort to identify compounds that could inhibit or even reverse the aggregation process should assess the effect of the potential inhibitors on the seeded aggregation of α -syn, among others. Considering that many potent anti-amyloidogenic agents have been isolated from Chinese herbal medicines (CHM), we screened thirty CHM compounds, for their effect on α -syn aggregation, seeded polymerization and toxicity by employing biophysical, biochemical and cell-culture-based techniques. Among the screened compounds, only ginsenoside Rb1, salvianolic acid B, dihydromyricetin and gallic acid were shown to be strong inhibitors of α -syn fibrillation, seeded aggregation and toxicity. Our results showed that gallic acid, ginsenoside Rb1 and salvianolic acid B inhibit α -syn fibrillation by binding and stabilizing the structure of the soluble, non-toxic oligomers, which are devoid of β -sheet content. In contrast, dihydromyricetin was found to be able to bind to both oligomeric and monomeric species of α -syn. In the case of gallic acid, the inhibition of α -syn fibrillation is related to the compound's hydroxyl moieties whose number and position on the phenyl ring were proven to be significant for the inhibition process, as indicated by the structure activity relationship data obtained from fourteen structurally similar benzoic acid derivatives. Overall, the compounds identified herein may represent the starting point for designing new molecules that could be utilized as drugs for the treatment of Parkinson's disease and related disorders.