1. No.

   2019G025

2. Title

   Drug discoverry targeting nicotinic acetylcholine receptor for neurodegenerative diseases with abnormal protein accumulation

3. Period

   2019-2023

4. Summary

Synucleinopathies are neurodegenerative disorders featured by the abnormal accumulation of α-synuclein (α-syn) protein. In this study, we focused on nAChRs as the therapeutic targets for synucleinopathies. We developed the *in vitro* and *in vivo* synucleinopathies model to analyze the effect of galantamine, which is an allosteric potentiating ligand of nAChR.

In *in vitro* study, α-syn pre-formed fibrils (PFFs) were generated from α-syn monomer by incubated with Dulbecco's PBS and agitation followed by sonication. SH-SY5Y cells overexpressing human α-syn (SNCA) gene were treated with PFFs or 24 h and then washed out. After the incubation for 48 h, immune-cytochemical analysis revealed the localization of α-syn. PFFs induced the intracellular aggregation of α-syn, which were detected with anti-phosphorylated-α-syn antibody and thioflavin-T. In the presence or absence of nonselective nAChR antagonist (mecamylamine, MEC), α4-nAChR antagonist (dihydro-β-erythroidine, DHβE) or α7-nAChRantagonist (methyllycaconitine, MLA), lysosomal inhibitor (NH4Cl) and chloroquine (CQ) α-syn PFFs-treated SH-SY5Y cells were incubated with galantamine. Galantamine treatment decreased the p62 expression, increased the LC3-II/I ratio and decreased the number of cells with intracellular α-syn aggregates. Galantamine-induced the promotion of autophagy flux and the inhibition of α-syn aggregation was suppressed by MEC, MLA, NH4Cl and CQ, but not by DHβE.

In *in vivo* study, PFFs were generated from α-syn monomer by incubated with K^+ free-157 mM Na^+ contain phosphate buffer, then microinjected into 20–24 weeks mice's right striatum. Mice were orally administered with galantamine (5 mg/kg, 1 time/day) in 12 weeks. PFFs microinjected mice were observed p-α-syn inclusions in brain, but there is huge difference in amounts of inclusion among individual mice. Therefore, significant effect was not observed between galantamine-administered mice and control mice.