Targeted drug discovery for Alzheimer disease: abridged secondary publication

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KEY MESSAGES

1. We identify a small molecule, ZL006, that possesses therapeutic effects by alleviating memory deficits in a mouse model of Alzheimer disease.
2. The therapeutic effects of ZL006 in enhancing learning and memory are likely due to its ability in blocking PTEN-mediated pathological events in synapses.
3. The mechanism of action of ZL006 is likely due to restoration of synaptic function but not by promoting neuronal survival.
4. ZL006 has the potential in treating early stage Alzheimer disease.

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Introduction

Alzheimer disease (AD) is an irreversible, progressive brain disorder that destroys memory and cognitive skills. The cumulative deposit of β-amyloid plaques and neurofibrillary tangles in the brain are considered the hallmarks of AD. The pathological mediator is a short 42 amino acids amyloid beta (Aβ) peptide. Aβ can perturb two forms of neuroplasticity. Indeed, enhanced long-term depression (LTD) and impaired long-term potentiation (LTP) accompanied by dendritic spine elimination and neuron loss could be elicited by acute Aβ treatment.[1] There are two classes of medication for AD: cholinesterase inhibitors and N-methyl-d-aspartate receptor inhibitors. However, the two types of drugs could only relieve the memory deficits in a very short period without any modification or delay in the progression of the disease.

Phosphatase and tensin homologue deleted from chromosome 10 (PTEN) is a lipid phosphatase with specificity towards phosphatidylinositol-3,4,5-trisphosphate. PTEN is a key negative regulator of the phosphoinositide 3-kinase/mammalian target of rapamycin signalling pathway.[2] The 403-amino acid PTEN protein harbours a PSD-95/Dlg1/ZO-1 (PDZ)-binding domain, which comprises four amino acids: Ile-Thr-Lys-Val. PDZ domain containing proteins often show interaction with the C-terminus of its binding partner PDZ-binding domain (PDZ-BD), especially in postsynaptic density (PSD). PSD-95 is one of the most abundant scaffolding protein involved in synaptic strengths and PSD architecture maintenance.

Previous studies have implicated PTEN involvement in N-methyl-d-aspartate receptor-dependent LTD, whereas basal synaptic transmission, LTP, mGluR-dependent LTD, and presynaptic paired-pulse facilitation are not affected.[3] The process of LTD induction is accompanied by the interaction between PTEN and PSD-95, which leads to the recruitment and anchoring of PTEN at the PSD in dendrite spines.[3] PTEN is recruited to the dendritic spines following treatment with Aβ. Moreover, this kind of recruitment is in a PDZ-BD-dependent manner. In other words, deletion of PDZ-BD results in the reduction in the level of PTEN recruited to the dendritic spines. Indeed, both Aβ-elicited abnormal LTD and LTP are abolished in the acute hippocampal slice derived from mice that lack PTEN PDZ-BD.[4]

We hypothesised that the blocking of the interaction between PTEN and PSD-95 could restore synaptic plasticity, and cognitive impairment would be alleviated in patients with AD. The aim of this study was to screen for compounds that can block the action of PTEN. Lead compounds would then be tested for their ability to alleviate cognitive impairments in a mouse model of AD.

Methods

Fluorescence polarisation

To screen for potential small molecule inhibitors, a fluorescent-labelled peptide FITC-PFDEDQHTQITKV-COOH was used as a fluorescent probe. A range of concentrations, 10 nM to 72 μM, of the fusion protein of GST-PSD-95 PDZ2

was incubated with 5 μM (for compound screening) or 10 μM (for standard curve) peptide for 30 minutes at 4°C. The extent of polarisation was measured on a SpectraMax i3 instrument. For compound screening, the compounds were added after the initial incubation, each mixture was incubated for 30 minutes at 4°C, the reaction mixture was assayed in the same way as for the standard curve.

Behavioural tests

Male 5XFAD transgenic mice aged 6 to 7 months were used as a model of AD. The Morris water maze test was performed at a pre-training day (day 0), 4 training days (days 1-4), and a probe trial day (day 5). The average of five trials was calculated to give a single path length and escape latency on each day for each mouse. For day 5, single values for the path length, escape latency, and time spent in the platform quadrant and tank for each mouse were collected. Data were analysed using two-way ANOVA.

Results

To identify small molecules that could inhibit the binding between PTEN PDZ-BD and the PDZ2 domain of PSD-95, we performed an in silico screening of a chemical library (ChemDiv; San Diego [CA], USA) of 4094 compounds with structural features that can dock the PSD-95 PDZ-2 binding pocket, and a docking score was calculated for each compound, with 60 highest-scored compounds selected for further validation. In addition, ZL006, which has been shown to disrupt ischaemia-induced nNOS and PSD-95 interaction, was also included for further investigation.[5]

Fluorescence polarisation is a biochemical assay for measuring the affinity of the interaction between a small ligand and its interacting protein of a greater mass. Fluorescent-labelled ligands in solution have a scattered emission profile when excited. However, binding to a bulky protein partner causes polarisation of the fluorescent emission, which can be detected by a spectrophotometer. Fluorescence polarisation assay was performed using GST-PSD-95 PDZ2 fusion protein and a 12-amino acid FITC-tagged peptide representing the PDZ-BD of PTEN. The standard binding curve was between GST-PSD-95 PDZ2 and PTEN PDZ-BD peptide with an EC50 around 18.26 μM. In contrast, the GST-PSD-95 PDZ1 domain has 30 times less binding capacity. We then tested the reference compound ZL006 at two concentrations at 25 and 50 μM with binding almost completely abolished at 50 μM. We constructed an inhibition curve for ZL006 and yielded an IC50 of 11.76 μM. Screening of 60 ChemDiv compounds identified four small molecules that possessed inhibitory activities but they were not pursued due to their weak inhibitory effects and undesirable toxicity in animals.

There was no toxicity in mice after administering ZL006 of up to 300 mg/kg. Owing to the potency of the inhibitory activity and apparent lack of toxicity, we decided to focus on ZL006 instead and performed a pull-down assay in mammalian cells. With the addition of ZL006, the interaction between full-length PTEN and GST-PSD-95 PDZ2 was largely suppressed in a dosage-dependent manner.

The Morris water maze test was performed to examine whether ZL006 has therapeutic effects on memory deficits in 5XFAD mice. Two groups of 5XFAD mice were treated with ZL006 (intraperitoneal, 10 mg/kg) for 30 minutes and 2 hours separately before the Morris water maze tests, with the treatment started from day 1 of the training session. Apart from the vehicle control, we treated one group of wild-type mice with ZL006 (intraperitoneal, 10 mg/kg) 2 hours before the tests to determine its effects on normal mice. In the pre-training day, there was no difference in visual and motor ability among the five experimental groups. During the training session, with the administration of ZL006, the five groups showed comparable velocity without significant differences indicating that ZL006 did not alter the motor functions in both wild-type and 5XFAD mice. Importantly, ZL006-treated 5XFAD mice showed shorter escape latency and path length on days 3 and 4 of the training session when compared to the vehicle-treated 5XFAD mice. The effect of ZL006 was evident as early as day 1 as demonstrated by the reduced escape latency. On probe trial day 5, mice treated with ZL006 for 2 hours had increased entries into the platform area and shorter latency to their first swim across the platform area. These findings suggest that ZL006 has therapeutic effects on memory deficits in 5XFAD mice.

Discussion

ZL006 was initially developed as a specific blocker targeting the interaction between nNOS and PSD-95, which forms under ischaemic condition.[5] The initial goal was to use ZL006 to lower the excitotoxicity associated with excessive glutamate receptor signalling. However, ZL006 could actually inhibit the binding between PTEN PDZ-BD and PSD-95 PDZ2 in fluorescence polarisation and in pull-down assays. We therefore concluded that ZL006 might be a potential compound for treating AD by disrupting the interaction between PTEN and PSD-95. ZL006 may restore synaptic plasticity by promoting LTP or suppressing LTD, or both. There was a lack of major adverse effects of ZL006 based on our toxicity test. We conclude that ZL006 may be effective in restoring normal synaptic transmission to neurons exposed to pathogenic Aβ at the early phase of AD.

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