Novel small molecule inhibitors of casin kinase 1 delta - A valid drug target for Alzheimer’s Disease

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Introduction

Alzheimer's Disease (AD) is a global healthcare issue driven by aggregating protein pathology that initiates decades before cognitive symptoms become evident. Currently available AD drugs only alleviate symptoms temporarily and amyloid-targeted therapies have failed to deliver improvements in these earlier stages of the disease. Recent developments in understanding the underlying basis of AD pathology point to the pathological role of hyperphosphorylated tau. In particular, revision of the classical pathology timeline suggests tau pathology initiates prior to amyloid dysregulation and is subsequently triggered by an AD phenotype to excess production of toxic amyloid. Significantly, it is now considered that beyond a certain point tau pathology will continue to propagate independent of amyloid.

Our previous studies identified casin kinase 1 delta (CK1δ) is an obligate requirement for generating hyperphosphorylated tau in Aβ. Protein (30-180) and mRNA (20-180) levels of CK1δ are highly upregulated in AD brain compared to normal, where it is most often found associated with paired helical filaments comprising hyperphosphorylated tau and it plays important roles in other features of AD such as cell cycle control, neutriic sprouting and circadian rhythm dysfunction further suggesting it as an important, multifactorial therapeutic target.

Using a combination of in silico modeling of the CK1δ ATP-binding pocket and in vitro screening we have developed a series of novel CK1δ inhibitors with nanomolar IC50 and good selectivity over a range of kinase anti-targets. Lead molecules selected from in vitro screens against CK1δ have been improved through multiple rounds of structure-activity relationship (SAR) profiling.

Materials & Methods

In silico modeling of CK1δ ATP-binding site
A proprietary computer model of the human CK1δ ATP binding site was modelled using crystal structures of yeast and rodent CK1δ homologs, human CK1γ (known and CK1δ inhibitors). The docking model was used to screen a library of c.13 million commercially available compounds using Proscope's ProteinScreen™ technology. Subsequent publication of the first human CK1δ crystal structure and SAR from in vitro testing of inhibitors validated the CK1δ binding site model and allowed further refinement.

In vitro testing for CK1δ Inhibitor Activity
CK1δ inhibition was determined in vitro using recombinant CK1δ, synthetic peptide substrates (20 mM) and ATP (10 mM) with each compound (10 mM). Results were reported as the percent residual activity. Subsequently, potent CK1δ inhibitors were tested for selectivity using a bespoke panel of 31 kinases including all human CK1 isoforms and predicted likely anti-targets. For compounds with promising selectivity, the IC50 was determined using the CK1δ assay as described above.

In vitro testing of CK1δ Inhibitor effect on tau phosphorylation
Human neuroblastoma-derived cell line SH-SY5Y expressing a double mutant form of human 2N4R tau (TMT7 cells, QPS, Graz Austria) were used to determine the potential to overexpress and subsequently treated with compounds PS110, PS278-05 or PS278-06 at all doses between 0.1 and 10 mM for 8 hours. Cells were then washed and harvested and tau protein extracts prior to analysis using the Tau pSer202 and y3 assay (Proteome Sciences plc, London, UK).

In vitro testing of CK1δ inhibitor effect on cognition and tau phosphorylation
Groups of 12 mice, male, age 12 months, SNAP-Tau mice, QPS, Graz Austria aged 8.5 months were dosed orally once daily at 30 mg/kg with vehicle, PS110, PS278-05 or PS278-06 for 8 weeks. Cognitive function was tested using the Morris Water Maze on days 50-54 and animals were then sacrificed 24h following last drug administration on day 56. For each animal the left and right cortex, hippocampus and rest of brain along with plasma were collected for analysis. For each the animal and right cortex, hippocampus and rest of brain along with plasma were collected for analysis. Tau protein was subsequently extracted from each brain sample and prepared into four fractions (cortex, hippocampus, cerebral cortex and kidney) in equal mass by extraction with cold 80% acetone. These fractions were subsequently processed with the Proscope’s ProteinScreen™ technology and in vitro testing of kinase activity using recombinant CK1δ (Proteome Sciences plc, London, UK).

Results

In silico design of potent & selective CK1δ inhibitors
A total of 409 potential inhibitors were selected from the initial screen with 1976 actually available for testing in vitro having >50% activity. A further 1976 SAP compounds maintained or improved activity. 1638 active compounds were tested in a panel of inhibitors and the IC50 against CK1δ determined for the most active and selective compounds (Figure 1).

In vitro effects of PS110 & PS278 on tau phosphorylation
Neither compound had a significant effect on TMT7 cell viability at any dose tested. A dose dependent reduction in phosphorylation at pS180T181 and pS202Thr205 (both CK1δ specific sites) was seen using the TMT-SRM assay (Figure 2).

In vivo effects of CK1δ inhibitor effect on cognition and tau phosphorylation
All compounds were well tolerated in this study with no overt signs of any adverse events. The TMT7 model shows cognitive decline from 5 months but not due to motor effects and performance in the Morris Water Maze was therefore used to study compound effect on spatial learning and memory. All compounds showed some improvement relative to vehicle-treated controls but only PS110 achieved statistical significance in both path length and escape latency (Figure 3).

Drug Metabolism and Pharmacokinetics
To investigate drug metabolism and ability to cross the blood – brain barrier samples of plasma, brain cortex, hippocampus and rest of hemisphere was analysed by LC/MS/MS. Both compounds showed relatively little metabolism after 2 hours in the main metabolites being N-Oxide/acetoxime derivatives (both compounds) and a demethylated compound (PS278-05). The parent molecule represented 70% (PS110) and 54% (PS278-05) of all species in plasma.

Discussion

Using a targeted proteomics approach and smart outsourcing strategy we were able to confirm CK1δ as a candidate tau kinase and generate our first lead compounds targeting nearly 500 small molecule inhibitors. Our two lead compounds PS110 and PS278-05 have been taken through to in vivo proof of concept studies in a mouse model of tau pathology (Figure 1). Administration of PS110 led to a significant improvement in spatial memory and leads to a reduction in tau phosphorylation.

References


Future Work

We continue to evaluate tau phosphorylation at specific amino acid residues in treated animals using tau TMT assays. Drug concentrations in the cortex, hippocampus and rest of brain as well as plasma levels and metabolites are currently being phosphorylation determined. Dependent on these results compounds may be tested in other rodent models of Alzheimer’s disease. 

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