Bioorganic & Medicinal Chemistry 26 (2018) 2859–2862

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Therapeutic peptides for CNS indications: Progress and challenges

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ARTICLE INFO

Article history:
Received 5 June 2017
Revised 5 September 2017
Accepted 8 September 2017
Available online 9 September 2017

ABSTRACT

Attacking neurodegeneration and promoting neuroprotection have been the holy grail in neurology for almost 20 years and represent an area of high unmet medical need. However, indications like Alzheimer's disease and stroke are areas in drug development fraught with failure. This review will highlight three CNS peptide programs which are tackling targets and indications in which traditional small molecule approaches have been difficult and challenging. The targets for these potential peptide therapeutics include the NMDA receptor, γ-secretase, and cyclin-dependent kinase in which direct inhibition has resulted in on-target (not compound related) problems. For example, direct inhibition of γ-secretase has resulted in gastrointestinal abnormalities and inhibition of the NMDA receptor can result in hallucinations, dizziness, out-of-body sensations, and nightmares. When confronted with show-stopping side effects, the CNS peptide programs profiled in this review strike the problem with intervention and disruption of selective protein-protein interactions. The goal of these peptide programs is to produce selective therapeutics with a better safety profile.

1. Introduction

Drug development is a fine balance between risk and benefit. Compounds need to be specific and selective for a particular target, have good metabolic properties and be able to get to their target. Getting to their target is an even greater challenge for CNS drugs because they need to be able to enter the brain which often requires crossing the blood-brain-barrier (BBB). During the period of 1995 to 2007, the approval rate for CNS drugs was 6.2% compared to 13.3% for non-CNS drugs.[21] A recent review conducted by the European Medicines Agency[4] interestingly found that of the 103 applications in neurology and psychiatry reviewed, short- and long-term safety as well as drug-drug interactions were problematic, areas in which peptides have the potential to excel in.

The challenges associated with CNS drug development begs the question as to whether alternative approaches need to be taken. For neurodegenerative diseases like Alzheimer's disease, numerous immunotherapy approaches have been considered to remove a pathological protein associated with the disease (see Ref. 25. The β-amyloid peptide (Aβ) has been a target for more than 10 years and several monoclonal antibodies have been tested in late-stage clinical trials. To date, there have been many failed studies with no clear clinical improvement.[9] Small molecule approaches in AD have also failed possibly due to a lack of specificity or the pleiotropic nature of the target. A reassessment or at least an alternative therapeutic tactic is needed to fill this high unmet medical need.

The molecular weight of peptides puts them between biologics and small molecules. The intermediate nature of peptides extends beyond just size. Small molecules can be design to be permeable and have large volumes of distribution in the body, but suffer from promiscuous properties like adverse, off-target and drug-drug interactions. On the other hand, biologics including therapeutic proteins and antibodies are known to be exquisitely selective and potent in terms of target engagement, often with picomolar affinities. However, the size of biologics is a disadvantage in terms of permeability and distribution.

Over 70 therapeutic peptides are on the market and another 150 in clinical development.[20] Peptides generally have very favorable pharmaceutical properties including high specificity and potency for their target, minimal potential for drug-drug interactions, lack of accumulation in tissues, and effectively metabolized by endogenous enzymes to non-toxic metabolites (namely the component amino acids). But peptides also have unfavorable pharmaceutical properties. Some challenges for peptide therapeutics include instability and short duration of action, inability to cross cellular membranes, and potential for immunogenicity. Recent research in peptide chemistry has made advances to resolve these challenges. For example, plasma stability can be prolonged by blocking the ends of the peptide with either *N*-acetylation or C-amidation to prevent exopeptidase degradation. The covalent attachment of a fatty acid or PEG not only protects a peptide from enzymatic degradation, but also reduce their elimination by the kidney. The covalent addition of various polymers can increase the molecular weight and hydrodynamic volume and thereby

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https://doi.org/10.1016/j.bmc.2017.09.011
0968-0896/

reduce renal clearance. Encapsulation of peptides in liposomes or degradable polymer matrices (e.g., PLGa) can also protect peptides from degradation and increase their circulating half-life.

To date, the target for most peptide therapeutic drugs have been extracellular receptors, since the hydrophilic character of the peptide bond makes cell permeability challenging. However, a CNS peptide therapeutic must be cell permeable in order to cross the blood-brain-barrier. Cell permeability can be engineered into peptides by the addition of a cell-penetrating peptide (CPP) sequence or macro-cyclization.[22] CPPs represents a family of small (< 30 amino acids), generally cationic peptides. At submicromolar concentrations, CPPs can transverse cellular membranes and can facility transport of a covalently conjugated cargo molecule. The cationic property of the CPP allows for association with the anionic cellular membrane. Although the mechanism for transport is not fully understood, both endocytosis and direct translocation are involved. CPPs not only have the potential to enable peptide therapeutics to access the brain, but also intracellular targets.

The high selectivity, specificity and potency of peptides to their targets poise them to tackle targets that are challenging, difficult or impossible to attack with either small molecules, due to the lack of selectivity, or by biologics, due to their size and lack of permeability. CNS peptide therapeutics have the potential to complement existing approaches to tackle very difficult targets and challenging indications.

Target: NMDA-glutamate receptors
Indication: Neuroprotection in stroke

Excitotoxicity is a proposed mechanism for ischemia-reperfusion induced neuronal damage. During conditions of hypoxia, excess glutamate is released which in turns results in activation of the NMDA-glutamate receptor with a flood of calcium and a vicious cycle begins resulting in apoptosis and cell death. Many neuroprotective strategies based on blocking the NMDA receptor have been attempted and to date, have failed.[24] These small molecule NMDA-receptor antagonists resulted in psychotomimetic sides effects including hallucinations, paranoid delusions, confusion, difficulty concentrating, agitation, alterations in mood, nightmares, catatonia, ataxia, anesthesia and learning-memory deficits. However, attenuation of the downstream NMDA-mediated signaling has great neuroprotective potential. At the synapse, the NMDA receptor subunits are in a macromolecular complex with the post-synaptic density protein PSD-95. This association is critical for mediating receptor-activated signaling events; therefore, uncoupling the NMDA receptor from PSD-95 is one approach to limit excitotoxicity. A peptide therapeutic would be well suited to disrupt protein-protein interactions, namely, the association or coupling of the NMDA receptor with the signaling complex protein PSD-95, and thereby be neuroprotective (see Fig. 1).

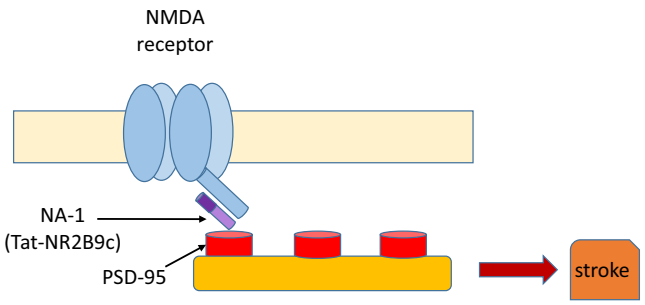

Fig. 1. Disruption of NMDA receptor coupling to PSD-95 by NA-1 peptide as a neuroprotective strategy in stroke.

A small nine amino acid peptide to the c-terminus of the NMDA 2B receptor (NR2B) can bind to the PDZ domain of PSD-95 and prevent interaction with NR2B. This peptide NR2B9c (Lys-Leu-Ser-Ser-Ile-Glu-Ser-Asp-Val) is exquisitely potent and selective, but in itself, is not cell permeant and cannot reach its intracellular target. A fusion construct of NR2B9c with the eleven-amino acid HIV-tat peptide confers cell permeability and distribution to the brain. The tat-NR2B9c fusion peptide is designated NA-1. A proof-of-mechanism study in rat confirmed the hypothesis that neuroprotection could be conferred in the MCAO rat stroke model.[1] Further validation of the proposed mechanism for neuroprotection was accomplished in a non-human primate model.[5,6] Macaques were subjected to middle cerebral artery occlusion (MCAO) to simulate stroke and administered either NA-1 or placebo. Improvement in several outcome measures were observed in the NA-1 treated animals, including a reduction in infarct volume and an improvement on the NHPSS functional outcome measure.[5,6]

As stroke is a difficult therapeutic indication to tackle, a novel approach to establish neuroprotection was taken by NoNO Inc (Etobiocoke, ON, Canada), the developers of NA-1 (tat-NR2B9c). Intracranial or cerebral aneurysms occur when a weakness in the wall of an artery or vein causes a ballooning of the blood vessel with a risk of rupturing and subsequent hemorrhage. One treatment for these aneurysms is endovascular coiling repair in which ~90% of the patients have small ischemic strokes cause by emboli released during the procedure. These small embolic strokes can be detected by diffusion-weighted (DWI) MRI and proof-of-principle for neuroprotection by the NA-1 peptide could be assessed.

A phase 2 clinical study investigated the safety and efficacy of NA-1 in patients undergoing aneurysm surgery (NCT00728182). A total of 197 patients were randomized with 185 treated with NA-1 or saline prior to undergoing aneurysm repair surgery. A statistically significant ($p = 0.018$) reduction in the number of DWI lesions was observed in the NA-1 treated group compared to placebo, although the volume of the lesions was not different.[17] This clinical proof-of-concept study demonstrated the potential for neuroprotection in the treatment of ischemic stroke.

Two phase 3 clinical trials are being conducted. FRONTIER (NCT02315443) is a multi-center, randomized, double-blind, placebo controlled study of intravenous NA-1 given within 3 h of symptom onset by paramedics in the field to patients with acute cerebral ischemia. The study is to recruit 558 patients and started in March 2015 with anticipated completion in 2019. A second Phase 3 study, ESCAPE-NA-1 (NCT02930018) is set to enroll 1120 patients with acute ischemic stroke undergoing endovascular thrombectomy and is expected to complete in 2020.

Target: Presenilin
Indication: Alzheimer's disease

Mutations in the presenilin protein have been identified for some familiar forms of Alzheimer's disease (AD). The presenilin protein is the catalytic subunit of γ-secretase, a key enzyme in the production of the toxic and pathological Aβ peptide in AD.[11] Aβ is produced in the brain by successive cleavage of the Amyloid Precursor Protein (APP) by γ- and β-secretase. The resultant 42 amino acid Aβ peptide can aggregate and form higher order oligomers which are neurotoxic. These aggregates of Aβ form the characteristic beta-amyloid plaques found in AD brains.

A working hypothesis in the treatment of AD is that reducing or removing beta-amyloid in the brain should modify or reduce the progression of the disease. There are currently a number of immunological approaches using monoclonal antibodies to various epitopes on amyloid plaques in late-stage clinical trials. An alternative approach to tackling beta-amyloid would be to prevent the

production by the inhibition of either β-or γ-secretase. Since APP is not the only substrate for these enzymes, preventing the processing of other proteins can lead to side effects. Several small molecule inhibitors of γ-secretase (GS) have progressed to clinical testing but failed due to severe gastrointestinal (GI) and dermatological adverse events (see Ref. 10). The GI side effect appears to be on-target inhibition of GS. The processing of membrane protein notch by GS results in the formation of the notch intracellular domain (NICD) which has important cellular functions in the GI tract.[16] Other small molecule approaches to reduce notch interaction but still inhibit GS have been problematic. Clinical development of ELND-006 was stopped due to liver toxicity, unrelated to GS inhibition.[18]

Safety is a paramount concern for small molecule inhibitors of GS. First generation small molecule inhibitors of GS targeted the aspartyl protease catalytic activity. These compounds lacked specificity both for GS but also for the substrate which GS processes (see Ref. 15). This is considered a target-related adverse pharmacology-toxicity and developing more selective or potent inhibitors are unlikely to circumvent the side effects. The next generation small molecule inhibitors of GS were designed to target an allosteric site on the enzyme and these compounds are referred to as γ-secretase modulators (GSM). Significant progress has been made in developing brain-penetrant and potent GSMs (see Ref. 8).

To circumvent some of the challenges associated with small molecule inhibition of GS, an alternative approach was devised by Dewji et al.[12] to disrupt the specific interaction between GS and the Alzheimer-selective substrate APP (see Fig. 2). A series of peptides based on the *N*-terminus of presenilin-1 were generated and binding to APP was confirmed by interferometry and fluorescent confocal microscopy.[12] Endogenous Aβ production was reduced by several of the peptides (P4, P6, P7 and P8). Once such peptide, P8 (Asp-Glu-Glu-Glu-Asp-Glu-Glu-Leu) reduced Aβ levels in the mThy1-hAPP transgenic mouse, suggesting the potential of P8 to be an AD therapeutic. Recently, it was shown that after intravenous, intranasal and subcutaneous administration, the P8 peptide crossed the blood-brain-barrier and was present in the brain and CSF.[13] The mechanism by which P8 crosses the blood-brain-barrier (BBB) is unknown; however, it is possible that P8 binds to presenilin located on the endothelial cells of the BBB which then facilitates entry into the brain. This hypothesis is under investigation. Cenna Biosciences (San Diego, CA, USA) is developing P8 as a treatment for Alzheimer's disease.

Target: Cdk5 kinase
Indication: Neurodegeneration (Alzheimer's disease, Frontotemporal dementia)

Cyclin-dependent kinases (Cdk) are a family of serine-threonine protein kinases that are expressed in proliferating cells and play an important role in the regulation of the cell-cycle. Cdk5 is a unique family member in that it is not involved in cell cycle regulation and is expressed predominantly in post-mitotic (non-dividing) neurons. The aberrant regulation of Cdk5 is known in several neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease (see Ref. 19. Cdk5 like the other cyclin-dependent kinases is regulated by various proteins in the signaling cascade. The CDK5R1 (p35) and CDK5R2 (p39) proteins are activators of Cdk5.[26]

CDK5R1 or p35 was the first activator of Cdk5 identified. The p35 protein is membrane-anchored and is composed of two domains, the *N*-terminal p10 and C-terminal p25. Binding of p25 to Cdk5 is required for activation, and an intricate regulation of Cdk5 involves autophosphorylation as well as ubiquitin-proteosome degradation (see review 26). Under various neurotoxic conditions, the p25 fragment is generated by proteolysis of p35 which leads to constitutive activation of Cdk5 in that the ability to turn off Cdk5 activity, which resides in p10 is absent in p25 (see Fig. 3). This interaction and activation of Cdk5 by p25 is believed to be a key pathological step in neurodegeneration.[19]

Cdk inhibition by small molecules have focused on interaction with the ATP-binding site. The conservation of amino acid sequence or homology between the ATP-binding domain of various Cdk-isoforms, makes selective inhibition challenging. It is important to note that Cdk5 is responsible for both normal and pathological functions in neurons. For example, Cdk5 activation regulates exocytosis, neuronal development & survival, and microtubule dynamics, necessary for retrograde and antegrade transport. Therefore, direct inhibition of Cdk5 is not desirable; however, under conditions of cellular stress like exposure to Abeta and/or neuroinflammation, Cdk5 can hyperphosphorylate tau and neurofilaments resulting in neurodegeneration and cell death (see Ref. 23). The key thereby requires therapeutic intervention to prevent the pathological pathways involving Cdk5 while leaving the normal function intact. This high degree of specificity and selectivity is a challenge well suited to peptide therapeutics.

The Cdk5 inhibitory peptide (CIP) is a 125-residue segment of p35 (amino acids 154–279) and found to bind at a higher affinity to Cdk5 than p25 and inhibits tau hyperphosphorylation.[28] Subsequent research on CIP found a smaller, 24-amino acid peptide (Lys-Glu-Ala-Phe-Trp-Asp-Arg-Cys-Leu-Ser-Val-Ile-Asn-Leu-Met-Ser-Ser-Lys-Met-Leu-Gln-Ile-Asn-Ala) which could specifically inhibit the "pathological" Cdk5-p25 activity without affecting the "normal" Cdk5-p35 activity.[27] To get this 24-amino acid peptide into the brain, Shukla et al.[27] added the 11-amino acid Tat cell penetrating peptide sequence (Tyr-Ala-Arg-Ala-Ala-Arg-Arg-Ala-Ala-Arg-Arg) to the C-terminus, creating a peptide referred to as TFP5. Recently, the macromolecular complex interactions of Cdk5, p35, and p25 suggests that the p10 region of p35 is important for proper localization of Cdk5 to its substrates,[2] and con-

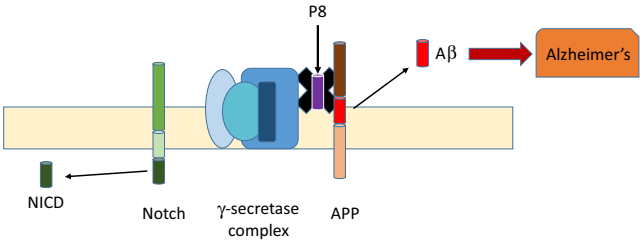

Fig. 2. Selective inhibition of γ-secretase:APP by P8 peptide to reduce Aβ production in Alzheimer's disease.

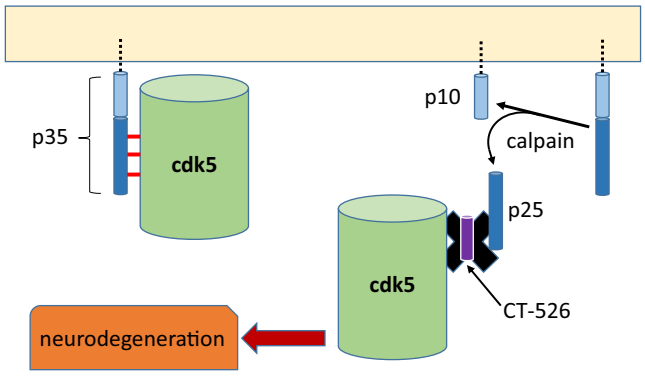

Fig. 3. Selective inhibition of Cdk5 activation by p25 by CT-526 (TFP5) peptide to prevent neurodegeneration.

firmed that TFP5 did not inhibit p35-activated Cdk5 when it was part of the endogenous macromolecular complex. CT-526 is a drug candidate based on TFP5 and is being developed by Cogentis Therapeutics (Baltimore, MD USA).

2. Summary

Stroke affects nearly 26 million people worldwide with approximately 10 million new strokes each year and 6.5 million deaths per year.[14] The only approved treatment for acute ischemic stroke is the use of alteplase which removes the occlusion. Many neuroprotective treatments have been tested with no success. Although the NMDA receptor has been strongly implicated in post-ischemic neurodegeneration, small molecule approaches to attenuate this receptor's pathological activity have been challenging due to the adverse side effects associated with blocking the receptor. A therapeutic peptide, NA-1 is in Phase 3 clinical trials and may provide the specificity necessary to elicit efficacy with a reasonable safety profile.

Likewise, the treatment of neurodegenerative disorders like Alzheimer's disease (AD) represents an area of high unmet medical need with the high rate of failure for new therapeutics. Alzheimer's disease is the sixth leading cause of death in the United States and there is an estimated 5 million Americans living with AD with the number increasing to ~16 million by the year 2050. Two preclinical peptide programs described in this review have the potential to address this unmet medical need, namely, P8 to inhibit the protease which produces the neurotoxic β-amyloid, and CT-526 to correct a dysfunctional protein kinase which results in hyperphosphorylation and neurodegeneration. In each case, the peptides target protein-protein interactions, which are particularly difficult interactions to disrupt with small molecules[7,3]).

CNS drug development is challenging in itself; however, if peptides can routinely and reliably reach their targets, CNS peptide therapeutics have the to potential to fill a niche of high unmet medical need. Additionally, peptides could help validate new targets for therapeutic intervention by providing mechanistic insight into the biology of CNS diseases.

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