Chapter

An Alternate View of Neuroprotection with Peptides in Alzheimer's Disease

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Abstract

Neuroprotection plays a crucial role in everyday life, maintaining a clean environment in the central nervous system to allow for normal functioning. In Alzheimer's disease and other neurodegenerative disorders, neuroprotection may have two roles. Under standard circumstances, the immune system protects the CNS, but sometimes it can exacerbate the pathophysiology of some diseases through neuroinflammation leading to further degeneration. Alzheimer's disease is fast getting out of control, with no new approvals in therapeutics since 2003, and of those approved, all target symptomatic treatment. Initiated by a microglial response to Aβ plaques, therapeutic development should focus on the amyloid cascade as a neuroprotective measure for Alzheimer's disease. This chapter will examine the status of the types of therapeutics in clinical trials for Alzheimer's disease, offering insights into peptides as an area of opportunity for neuroprotection and detailing considerations for the use of peptides in Alzheimer's disease.

Keywords: Alzheimer's disease, peptides, neuroinflammation, therapeutic development, CNS indications

1. Introduction

The central nervous system (CNS) consists of the brain and spinal cord, playing the role of control centre in the body. It is responsible for sending and integrating signals from around the body and coordinating activity. Protecting the CNS is crucial to sustaining life. Without this system, normal day-to-day functions such as breathing and eating would be compromised. Arguably, the most important organ in the CNS is the brain. This is protected from external physical injury by the skull and meninges, which provide a buffer against forceful trauma to the head. How does the brain protect itself from internal injury, such as a microbiological threat or other small molecules that invade the sterile environment? Bacteria, viruses and misfolded proteins are as much of a threat as physical impacts. However, there is no durable exterior to protect from these internal attacks. The next line of defence is the immune system, a complex network of specialised cells that aim to protect the body against these biological threats.

The immune response is key to maintaining the delicate environment of the CNS. However, the neuroprotective properties of the immune system may also be detrimental to the surrounding neurons. Immune cells release chemical mediators

such as cytokines and histamine to damage foreign cells, but these mediators also damage sensitive structures that make up the brain. This process occurs in disorders where degeneration of cellular tissue in the brain is present. Disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Amyotrophic Lateral Sclerosis (ALS) all exhibit progressive degeneration of specific neuronal cell populations [1, 2]. All four diseases are commonly found to exhibit misfolding, aggregation and accumulation of specific proteins. This hallmark feature is now widely accepted as a possible cause for these diseases and other neurodegenerative disorders [3–5]. Deposition of amyloid-forming proteins functions as the initiating step for neuroinflammation [6] activating pattern recognition receptors (PRRs) on microglia, the resident macrophages in the brain [6, 7]. To protect the brain, microglia recognise fragments of these misfolded proteins and secrete cytokines and chemokines. The release of these pro-inflammatory immunomodulators mediates neuroinflammation, attracting other immune cells such as astrocytes and perivascular macrophages to aid in innate immunity [8]. In most cases, activated microglia will clear the build-up of the pathogenic proteins resolving the immune response and subsequent inflammation.

In a typical immune response where resolution is achieved, clearance of the localised inflammation allows the surrounding tissue to return to normal conditions. When the immune response is not resolved, inflammation persists in the local area, potentially becoming toxic to neighbouring cells. Prolonged inflammation in a sensitive environment such as the CNS is highly likely to cause damage to neurons and other nearby cells, leading to local degeneration of tissue. Damage-associated molecular patterns (DAMPs) released from neurons in the inflamed area are recognised by PRRs on primed microglia. This further stimulates the release of pro-inflammatory molecules [9]. This persistent self-propagating cycle of inflammation and necrosis causes the chronic inflammation that exacerbates the pathology of the disease. The notion that neuroprotection does more damage than it prevents has been explored recently, with some proposing that inflammation is the causative agent of neurodegeneration [10, 11]. To prevent neurodegeneration found in diseases like AD, neuroprotective therapeutics must be developed in order to prevent further inflammation and damage from occurring.

1.1 Alzheimer's disease as a neuroinflammatory disorder

Alois Alzheimer first discovered clusters of abnormal protein built up in the cerebral cortex of a patient in 1906. Alzheimer described these clusters as "thick bundles [that] appear at the surface of the cell", noting specifically that neurons in the upper layers of tissue had "disappeared" [12]. These bundles were later identified as the two major hallmarks of AD, hyperphosphorylated tau and aggregated amyloid-beta (Aβ). Alzheimer also noted glial cells clustered around the plaques, concurrent with the theory of an immune response to the extracellular deposits of Aβ plaques and cellular death. In 2019, we are still no closer to mapping out the nature of this disease than Alois was in 1906, with the pathophysiology of the disease still debated: which came first, the tau or the plaques? There have been several hypotheses considered over the nature of the disease; however, the two major hallmarks remain the most probable causes.

To describe the basis of the two major hypotheses is easy; the amyloid cascade involves the cleavage of a transmembrane protein known as amyloid-precursor protein (APP) by the aspartic-acid protease beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), which leads to the extracellular aggregation of a peptide called Aβ, whereas the neurofibrillary tangle (NFT) theory posits that AD

is caused by the hyperphosphorylation of tau, a soluble microtubule-associated protein that can aggregate intracellularly into NFTs.

Before establishing the effects of a therapeutic for neuroprotection in AD, the causative agent of neuronal death needs to be identified. Examining both hypotheses in detail reveals that the deposition of Aβ plaques has more of an effect on NFT formation than hyperphosphorylation of tau has on amyloid build-up [13, 14]. Arguments for both options are common place in discussion about AD; however, there are some key facts on why Aβ plaques are crucial in the development of neurodegeneration, and therefore the symptoms of AD. In transgenic mouse models, it has been shown that NFT formation succeeds Aβ deposition extracellularly [15, 16]. As a causal agent, tau is seen in other diseases such as frontotemporal lobar degeneration, progressive supranuclear palsy and Pick's disease. All of which form tau aggregates without an onset of Aβ deposition. The distinct immune response from Aβ plaque deposition indicates that the amyloid cascade is the driving factor of neurodegeneration in AD [10]. From a neuroprotective standpoint, preventing the amyloid cascade from generating and depositing Aβ plaques seems the most probable option for prevention of neurodegeneration from chronic neuroinflammation.

2. Therapeutics for Alzheimer's disease: past, present and future

In a 20-year period from 1998 to 2017, a total of 146 drugs in clinical trials were halted or had not received approval by the FDA [17]. In that same time, four cognitive-enhancing therapeutics had been approved, giving some hope that there is a chance to identify a therapeutic for AD. Therapeutics in the AD clinical trial pipeline are split into two major classes of mechanism of action (MOA): symptomatic treatments and disease-modifying therapies (DMTs). Symptomatic treatments aim to alleviate symptoms that are present with the onset of the disease easing the burden on the affected individuals. There are currently five therapies that have been approved for use in patients that exhibit symptoms derived from neurotransmitter disturbance in mild to severe cases of AD. Suppressing symptoms such as memory loss and cognitive decline do not address the underlying nature of the disease [18]. Symptomatic treatments are beneficial for family and friends, demonstrating modest and consistent benefits for cognition. However, the underlying cause of the disease remains unchanged in these therapies where the disease progresses into a more severe state. DMTs are treatments that alter the pathology of the disease, changing the long-term course of the disease. A large proportion of DMTs targets the major hallmarks of AD, NFTs and Aβ formation. Other DMTs are present that target alternative aspects of the disease; however, these alternative targets are mostly downstream effects of NFTs or Aβ plaques. Of major interest are DMTs that target the amyloid cascade, their primary goal is to reduce plaque load, clear plaque depositions, or reduce inflammation. The nature of this MOA is of a neuroprotective stance, theoretically with the ability to reduce the amount of neurodegeneration that occurs due to chronic inflammation from Aβ seeding in the extracellular space.

As of February 2019, 132 therapeutics were in clinical trials for AD, 96 of those classed as DMTs presenting an increase of 25 DMTs from 2018 [19, 20]. Therapeutics labelled as neuroprotective, anti-inflammatory and anti-amyloid in the 2019 cohort of clinical trials will be described as neuroprotective DMTs as they all target the amyloid cascade as the priming step of neuroinflammation. Neuroprotective DMTs are described as either prophylactic treatments or disease-clearing treatments. Prophylactic treatment of AD aims at preventing the onset

of the disease by targeting the steps prior to amyloid deposition aiming to prevent the activation of microglia and subsequent neuroinflammation. Disease-clearing therapeutics target plaques deposited into the extracellular space. They focus on removing plaques and debris to prevent chronic inflammation. There is no clear current trend in neuroprotective DMTs, with a broad selection of therapeutics covering different targets from amyloid clearance using antibodies or vaccines to mark areas for the immune system, anti-aggregation of Aβ fibrils, or preventing the production of Aβ fragments by targeting BACE1 or alpha secretase.

2.1 Lessons from previous clinical trials

With such a broad range of therapeutics in clinical trials, it would be easy to assume that we are close to finding a treatment for AD, but we are not. In the 20 years spanning 1998 to 2017, almost 150 therapeutics in clinical development had stopped or not received regulatory approval [17]. The FDA approved only four therapeutics in that time leaving a lot to learn from past failures. Neuroprotective DMTs made up 34% of the therapeutics discontinued in this time, leaving in their wake a plethora of lessons that can be applied to upcoming therapeutics [21]. A shift in development from the conventional small molecule drug (SMD) to a biological approach has shown benefits. Increased knowledge on the effects of more potent and specific therapeutics has led to the identification of new targets for therapeutic development, specifically the amyloid cascade. Of the therapeutics active in clinical trials in the 15 years from 2005 to 2019, 79 targeted the amyloid cascade in a disease-modifying mechanism (**Table 1**). Moreover, of the 79 clinical trials, 20 have been discontinued (**Table 1**).

2.1.1 Types of therapeutics

A shift in the type of therapeutic used in AD has given insights into how targets respond to certain molecules. A common issue encountered with amyloid targeting therapeutics is specificity, with off-target effects halting a few large-scale trials [22]. There are two major molecular classes present in amyloid targeting DMTs: small molecule, low molecular weight entities including chemical drugs and peptides, and biologics, larger structures such as proteins and antibodies.

2.1.1.1 Small molecular entities

Thought of as the traditional form of therapeutic, small molecular entities (SMEs) are typically chemical in nature and mostly target molecules with deep catalytic channels or clefts such as enzymes or receptors [23]. The nature of these SMDs is to bind to the target and exert its effect, doing so until there is no more target available for binding or the drug is cleared from the body. This overzealous technique of SMDs poses the risk of long-term modulation on the target, whether it be positively or negatively, regardless of whether the disease state improves or not [24].

The main target of an SME is commonly found in biological processes where a high amount of regulation is required, in the form of either enzymes or receptors [25]. The interaction that SMEs target is between an enzyme or receptor and its respective substrate, all of which are proteins. Referred to as protein–protein interactions (PPIs), they have gained popularity as a target for therapeutic intervention due to the control these interactions have on biological processes. Many PPIs have been identified as candidates targeting diseases similar to AD where a biological process has been altered resulting in disease [25].

NCT number	Drug name	Phase	Status	Start date	Completion date
NCT00303277	Simvastatin & Pravastatin	IV	C	08/2002	04/2005
NCT00479219	GSI-953	I	C	05/2007	10/2007
NCT00765115	LY450139	I	C	07/2006	09/2007
NCT0083808	LY2811376	I	C	12/2008	06/2009
NCT00733642	PF-04360365	I	A, NLR	08/2008	07/2009
NCT01125631	PF-04360365	I	C	05/2010	08/2011
NCT01148498	Solanezumab	II	C	08/2010	08/2012
NCT01482013	HPP854	I	D	10/2011	03/2012
NCT00464334	V950 and ISCOMATRIXTM	I	C	03/2007	01/2012
NCT00411580	CAD106	I	C	06/2005	12/2008
NCT00945672	PF-04360365	II	C	08/2009	06/2011
NCT01547169	Insulin detemir	II	C	03/2011	12/2012
NCT00500500	EGb 761	II	D	07/2005	04/2008
NCT00739037	PAZ-417	I	D	08/2008	12/2008
NCT01568086	Affitope AD03	I	D	12/2011	10/2013
NCT01661673	EVP 0962	II	C	11/2012	10/2013
NCT00812565	Immune Globulin	II	C	02/2009	09/2010
NCT00857506	Florbetapir F 18	II	C	01/2009	12/2011
NCT00397891	Bapineuzumab	I	C	10/2006	02/2010
NCT01035138	Semagacestat	III	C	12/2009	04/2011
NCT01669876	Anatabine	II	D	08/2012	02/2015
NCT01978548	Atabecestat	I	C	12/2013	04/2015
NCT02061878	Bexarotene	I	C	08/2014	11/2014
NCT00486044	Simvastatin	II	C	02/2005	06/2009
NCT00711321	Affitope AD02 & Aluminium hydroxide	I	C	11/2008	04/2010
NCT01093664	Affitope AD02 & Aluminium hydroxide	I	C	10/2009	07/2010
NCT01357629	Affitope AD02 & Aluminium hydroxide	I	D	07/2011	11/2013
NCT00633841	Affitope AD02 & Aluminium hydroxide	I	C	02/2008	09/2009
NCT01782742	Bexarotene	II	C	02/2013	12/2014
NCT02323334	LY3202626 & Itraconazole	I	C	12/2014	02/2016
NCT00722046	Ponezumab	II	C	12/2008	08/2011
NCT00956410	Amilomotide	II	C	09/2009	06/2011
NCT00762411	Semagacestat	III	C	09/2008	04/2011
NCT01097096	Amilomotide	II	C	03/2010	12/2012
NCT01928420	Pinitol	II	C	04/2007	06/2014
NCT00329082	Solanezumab	II	C	05/2006	05/2008

NCT number	Drug name	Phase	Status	Start date	Completion date
NCT01600859	Elenbecestat	I	C	07/2012	10/2013
NCT01297218	hMSC Therapy	I	C	02/2011	12/2011
NCT01193608	AAB 003	I	C	09/2010	10/2013
NCT02260674	Atabecestat	II	C	11/2014	06/2016
NCT02033668	GSK 933776	I	C	01/2014	07/2014
NCT01424436	GSK 933776	I	C	05/2010	12/2011
NCT02576639	Umibecestat	II	C	08/2015	03/2016
NCT00904683	Solanezumab	III	C	05/2009	06/2012
NCT02386306	GC 021109	I	C	02/2015	10/2015
NCT01595646	Insulin detemir	II	C	11/2011	03/2015
NCT01561430	LY 2886721	I/II	D	03/2012	Jun 2013
NCT02551809	UB 311	II	C	10/2015	08/2018
NCT03417986	Thiethylperazine	II	A, NLR	11/2017	07/2021
NCT01056965	Davunetide	I	C	01/2010	12/2012
NCT01428453	Rilapladib	II	C	07/2011	02/2013
NCT02036645	MEDI 1814	I	C	02/2014	09/2016
NCT01397578	Crenezumab	II	C	07/2011	04/2014
NCT01127633	Solanezumab	III	D	11/2010	02/2017
NCT02760602	Solanezumab	III	D	06/2016	05/2017
NCT01900665	Solanezumab	III	D	07/2013	02/2017
NCT02080364	Azeliragon	III	D	04/2015	06/2018
NCT01807026	LY 2886721	I	C	03/2013	05/2013
NCT02462161	Insulin aspart	I	C	03/2015	04/2019
NCT02899091	CB-AC 02	I/II	R	09/2016	12/2021
NCT02614131	LY 2599666 & Solanezumab	I	D	12/2015	12/2016
NCT02406027	Atabecestat	II	D	07/2015	06/2018
NCT02051608	Gantenerumab	III	A, NLR	03/2014	04/2021
NCT03114657	Crenezumab	III	D	03/2017	06/2019
NCT02565511	Amilomotide & Umibecestat	II/III	D	11/2015	03/2025
NCT01760005	Atabecestat & Gantenerumab & Solanezumab	II/III	D	12/2012	03/2021
NCT01224106	Gantenerumab	III	A, NLR	11/2010	08/2020
NCT01966666	TPI 287	I	A, NLR	11/2013	11/2019
NCT00594568	Semagacestat	III	C	03/2008	05/2011
NCT02719327	E-EPA	II/III	R	06/2017	11/2021
NCT02956486	Elenbecestat	III	D	10/2016	11/2023

NCT number	Drug name	Phase	Status	Start date	Completion date
NCT03036280	Elenbecestat	III	D	12/2016	11/2023
NCT02245737	Lanabecestat	II/III	D	09/2014	10/2018
NCT03443973	Gantenerumab	III	R	08/2018	03/2023
NCT03444870	Gantenerumab	III	R	06/2018	03/2023
NCT00299988	Immune Globulin	II	D	02/2006	04/2010
NCT02600130	hMSC Therapy-Longeveron	I	A, NLR	10/2016	09/2020
NCT03402659	Neflamapimod	II	C	12/2017	07/2019
NCT03117738	Adipose SC therapy-Anterogen	I/II	C	04/2017	06/2019

Abbreviations: A, Active; NLR, no longer recruiting; C, Completed; D, Discontinued. NOTE: 79 trials were identified as amyloid targeting as of January 9, 2020, according to https://adisinsight.springer.com.

Table 1.
Amyloid targeting clinical trials from 2005 to 2019.

Analysis of SMEs targeting PPIs has shown that they do not observe standard drug-like properties, specifically surrounding their size, hydrophobicity and specificity [26, 27]. These properties all show mild increases, compared to conventional SMDs, proving that the chemistry of PPIs requires molecules to be selected more carefully rather than selecting the molecule with the highest potency.

2.1.1.2 Biologics

Biologics fill the void of the upper end of the molecular weight scale, made up of antibodies, proteins and enzymes. Biological therapeutics like antibodies and vaccines aim to modulate the immune system to clear various threats from the body. Other therapies involve the replacement of an important molecule in a biological process, such as hormone replacement therapy (HRT) or lactose intolerance. Replacement therapies use therapeutics that mimic proteins in a healthy individual, usually using recombinant technology to produce the protein in different biological models.

The PPIs mentioned above have important regulatory roles in biological processes, keeping them in check as cell signalling molecules [28]. Biological intervention with molecules that mimic or stop these interactions enables control over biological pathways similar to SMDs, however, giving the pathway some control over feedback [24]. Antibodies are an excellent example of controlling the immune system in AD to remove the build-up of deposited plaques, while allowing the body to exert control over the reaction of the immune response to these antibodies. This shows the benefit of using biologics in the development of therapeutic options for AD and other diseases.

A common issue that has arisen in the therapeutic development of biologics is the bioavailability and half-life of the therapeutic. Biologics are not well known for high bioavailability, particularly where the oral route is concerned, an issue that can be overcome using other forms of administration [29]. Following administration, biologics are subjected to proteases and harsh conditions in the stomach or other accessory organs that reduces the half-life dramatically [30]. Intravenous and intramuscular administration has improved the half-life of biologics; however,

modification to the structure of the therapeutic may be required to reach the target from the blood stream or the tissue at the site of injection. Although there are common issues regarding ideal therapeutic properties of biologics, new technology is improving every day allowing therapeutic development of biologics to overcome what seems to be simple obstacles.

2.1.2 Targets of the amyloid cascade

2.1.2.1 Inflammation

As the step preceding neurodegeneration, inflammation is a clear target for therapeutic intervention. Neuroinflammation has been a target in therapeutic development after the increasing recognition that glial activation is an important step in the process of neurodegeneration. Therapeutics targeting neuroinflammation have been evaluated in previous cohorts of clinical trials, all producing similar results of ineffectiveness at slowing cognitive decline [31]. The principle of anti-inflammatory targeting aims to prevent the off-target damage to neighbouring neurons, delaying the onset of neurodegeneration. However, without removing the stimulus causing neuroinflammation, therapeutics are fighting a losing battle. Factors such as rate of cognitive decline, severity of the neuroinflammatory response, and disease state play a role in anti-inflammatory and immunomodulatory drug discovery proving difficult as the timing of the intervention is critical [32]. Anti-inflammatory therapeutics made up 16% of therapeutics in clinical trials in 2019, many repurposed for AD [19]. As a sole therapeutic intervention for AD, anti-inflammatories are not ideal. Combined therapy using anti-inflammatories and a disease-clearing therapeutic will likely show a high rate of success in alleviating chronic inflammation caused by AD.

2.1.2.2 Amyloid-β

Aβ is an attractive target for neuroprotection, with many possible angles to approach the underlying cause of the disease. Two methods aimed at targeting Aβ plaques have been employed in clinical trials: immunotherapy, targeting the plaques for removal by the immune system using a vaccine or monoclonal antibodies (mAbs), and anti-aggregation, preventing the Aβ fragments from forming the plaques. In 2019, nine immunotherapies were part of clinical trials: three active immunotherapies (vaccines), CAD106, ABvac40 and GV1001, and six passive immunotherapies (mAbs), aducanumab, crenezumab, gantenerumab, solanezumab, LY3002813 and LY3372993 [19].

Thoroughly tested in animal models, Aβ vaccines exhibit the ability to prevent the formation of new Aβ plaques and contribute to the clearance of pre-deposited plaques [33]. Immunising an individual to Aβ grants the long-term effects of antibody production. However, immunisation can be difficult where adverse reactions in older individuals may occur due to inconsistent or lack of an immune system, as well as selecting a specific epitope that will not target similar structures [34]. Clinical trials into AN1792, an adjuvant vaccine of the full-length Aβ peptide and QS-21, were stopped due to development of meningoencephalitis in some patients [35]. Second-generation anti-Aβ vaccines such as CAD106 have proven to be efficacious in phase 2 clinical studies, eliciting Aβ-specific antibodies and showing long-term safety promising to be a valuable therapeutic option [36].

Passive immunotherapies have a major advantage over Aβ immunisation in that there is a consistent antibody titre [34]. Initial intravenous administration

of immunoglobulin preparations containing high levels of human anti-Aβ42, which showed a significant improvement in cognition and lower levels of Aβ [33]. However, similar to the other discontinued anti-Aβ mAb therapies, large-scale testing proved efficacy to be low or non-existent. A risk found in trials with Bapineuzumab was the presence of abnormalities after imaging the brain, identifying the onset of vasogenic oedema in 3 of the 10 participants. These abnormalities were coined as ARIA-E, amyloid-relating imaging abnormalities-vasogenic effusions, and are seen as a risk in large-scale studies of mAb therapies [37]. Many mAbs in 2019 are still plagued with these obstacles, presenting safety concerns surrounding ARIA-E, although some mAbs in Phase 2/3 or 3 trials are looking closer than ever at slowing the progression of AD.

As a target class, combined therapy of immunotherapeutics and anti-aggregates stand the highest chance of clearing deposited and newly generated Aβ fragments. Aggregation of Aβ monomers only make it more difficult to clear from the extracellular space with neuroprotective mechanisms naturally clearing monomers that build-up over time. From this perspective, Aβ is targeted as both monomers and plaques. Solanezumab targets Aβ monomers before they can aggregate. Targeting the causal feature of amyloid-based microglial activation, anti-aggregates prevent the conversion of Aβ monomers into oligomers or fibrils [38]. Many natural and synthetic compounds have been identified as potential anti-aggregates for Aβ; however, the only anti-aggregate for amyloidogenesis in clinical trials in 2019 is a combination therapy of polyphenol extract from grapeseeds and resveratrol [19]. The current cohort of anti-aggregates is not indicative of knowledge of the field, with other compounds such as epigallocatechin-3-gallate and curcumin showing promising results for both anti-aggregation and other purposes [39].

2.1.2.3 Secretase inhibitors

Modulating the upstream step of plaque formation provides an encouraging target as prevention of deposition of Aβ fragments may stop the neuroinflammatory response before it starts. A "one size fits all" therapy for secretase modulation is not possible as all three secretase enzymes play different roles in the generation of Aβ fragments, each requiring a different form of modulation specific to their role in the processing of APP.

2.1.2.3.1 Gamma secretase

Inhibitors of BACE1 and gamma secretase have thus far showed limited Aβ clearance in clinical trials, even after demonstration of excellent inhibition in preclinical animal models [40]. Studies into gamma secretase found that it was the last step in Aβ fragment generation and an ideal target to prevent the build-up of fragments and formation of plaques [41]. Semagacestat was identified as potential drug candidate for clinical trials in decreasing Aβ levels, only after Phase III in the IDENTITY trials was it found to have adverse effects on Notch signalling [42]. Identified as a drug with a higher selectivity for APP over Notch in preclinical studies, Avagacestat was another gamma secretase inhibitor that showed similar effects to Semagacestat forcing the discontinuation of the trials due to adverse dose-limiting effects [43]. The adverse effects and lack of efficacy had quashed further research into gamma secretase inhibitors; however, a new look into gamma secretase as a target has identified that it is available for modulation, specifically altering the cleavage site of the enzyme. NGP 555 is a promising SME gamma secretase modulator that has showed promising results *in vivo,* significantly lowering levels of $A\beta_{42}$ through a shift of cleavage site in gamma secretase [44].

2.1.2.3.2 Alpha secretases

Alpha secretase as a therapeutic target for AD offers a novel approach of upregulating cleavage of APP rather than preventing it. By cleaving APP within the Aβ domain, alpha secretase prevents the generation of Aβ fragments instead releasing non-toxic p3 peptide following gamma secretase cleavage [45]. Modulation of alpha secretase is expected to increase its activity and reduce levels of Aβ, potentially increasing the levels of a neuroprotective product of alpha secretase cleavage of APP, sAPPα [46]. Alpha secretases belong to the 'a disintegrin and metalloprotease' (ADAM) family, which are found to play roles in cell adhesion, migration, proteolysis and signalling [47]. ADAM10 was found to be the alpha secretase relevant to APP cleavage in neurons, making it the target of modulation in AD [48]. Two therapeutics that have undergone clinical trials showing potential as alpha secretases enhancers are etazolate (EHT-0202) and bryostatin-1. Both stimulate alpha secretase to increase generation of sAPPα [49]. The potential of alpha secretase enhancers as a therapeutic for AD is likely. However, studies into the effects of enhancers on the other substrates of ADAM10 are required to identify any possible adverse effects [50].

2.1.2.3.3 BACE1

Targeting BACE1 for therapeutic development in AD is ideal, as it is the determining step in the generation of Aβ fragments. Inhibition of BACE1 has shown to decrease levels of Aβ plaques. Studies in mouse models have proven that by removing BACE1 there is no generation of Aβ fragments, and subsequently no neurodegeneration and loss in cognitive abilities [51].

Since it was discovered to play a role in AD in 1999, BACE1 has been thoroughly researched as a potential target for AD. BACE1 has been an elusive target for inhibitors, its location in the brain, size of the active site, and similarity to other aspartic proteases making it difficult for the ideal therapeutic to be developed [52]. Initial inhibitors of BACE1 were non-cleavable peptide-based analogues, designed on the amino acid sequence of APP, which showed excellent inhibitory effects on BACE1. However, the size was too large to exhibit *in vivo* benefits, although ideal for the active site [53]. The development of SMEs for BACE1 renewed hope in the use of the aspartic protease as a target, hoping to increase blood–brain barrier (BBB) penetration and bioavailability that were identified as issues with the first-generation BACE1 inhibitors. From there, the hunt for a BACE1 inhibitor began with multiple classes of inhibitors being developed in an attempt to find the ideal therapeutic.

In a similar pattern to other amyloid therapies, BACE1 inhibitors in other trials were halted or discontinued due to lack of efficacy or off-target effects. Only two BACE1 inhibitors were in the 2019 cohort of clinical trials: CNP520, discontinued in July 2019 due to worsening of cognitive function, and E2609, discontinued in September 2019 due to unfavourable risk–benefit ratio [54, 55]. Both compounds joining the list of lessons learnt from BACE1 inhibitors, along with Lanabecestat, Atabecestat and Verubecestat. All of which proved excellent in reducing Aβ; however, translation into clinical trials was not as smooth, lacking efficacy or displaying off-target effects [56].

2.2 Improving therapeutics or target choice

With no current curative treatments for AD available, previous cohorts of clinical trials are missing something vital. The types of therapeutics used and targets

available explained above show that therapeutic discovery is not a simple task, particularly in a disease as complex as AD. The ideal neuroprotective therapeutic for combating AD is one that targets the initiating steps of amyloid development with high specificity and potency, while not disrupting other biological processes. An attractive initiating step of amyloid development is BACE1, discussed above as a promising target to prevent the generation of Aβ fragments responsible for the activation of microglia and subsequent development of neurodegeneration. Previous attempts at inhibiting BACE1 have shown mixed and unfavourable responses of properties such as specificity, bioavailability and efficacy. Both biologicals and SMEs cannot fill the requirements of such a specific therapeutic, requiring a molecule that has the ideal properties of both. Such a molecule is already being explored in therapeutic development for AD although it is still in its infancy as a class of therapeutic molecule for AD. Peptides are becoming more attractive as a therapeutic to target BACE1 with new technology altering their structure to better fit the required properties. Such research promises to pave new and exciting ways to developing refined peptide inhibitors of BACE1 with high efficacy and specificity, and thus prepare novel reagents for the prophylactic treatment of AD in the near future.

3. New outlook for Alzheimer's disease

3.1 What are peptides?

Peptides are small molecular biologicals that play a major role in the body as signalling molecules. Naturally occurring peptides in humans are commonly called hormones, acting as messengers utilising the blood stream and other extracellular spaces to regulate the many biological processes that keep us going [57]. Two of the most well-known peptides are glucagon and insulin, both playing large roles in homeostasis of blood-glucose levels. These hormones act on blood-glucose levels by targeting accessory organs and stimulating glucose production or glycogen storage, respectively. The action of glucose and insulin is a classic example of how peptides work in the body with high specificity and rapid onset of effect. Although commonly linked to hormones, peptides are also used as neurotransmitters, anti-infectives and growth factors [58].

Peptides range in length from 10 to 50 amino acids long, and can have a mass of up to 5 kDa, putting them between SMEs and proteins in terms of size and weight. *In vivo*, natural peptides are highly efficacious and selective with limited off-target effects, transient at most for those that exist [59]. Their ability to act as signalling molecules both extracellularly and intracellularly displays the range of therapeutic opportunity that peptides exhibit. Following the discovery of peptides playing large roles in homeostasis in the body, research turned towards identifying and isolating certain peptides that were linked to diseases. To continue with the example of insulin, the development of insulin as a therapeutic comes from the identification of individuals lacking a "pancreatic secretion" in the early 1900s, where insulin was isolated from the pancreas of stray dogs and calves and used to treat a child with type I diabetes [57]. This discovery only fuelled the fire for further discovery and isolation of other natural peptides that were found to be involved in diseases, leading to the identification of over 7000 naturally occurring peptides. Although identified, not all can be used directly as a therapeutic due to unbeneficial properties such as poor bioavailability and short half-life [58].

3.2 Peptides as therapeutic options

The nature of tasks that peptides perform in the body makes them an enticing molecule, as an opportunity to control biological processes in a similar way that hormones and other natural peptides control everyday life. Many consider peptides to be the inferior option for therapeutic development as they display low oral bioavailability and a tendency to be metabolised by proteolytic enzymes in the local environment leading to a short half-life *in vivo* [57]. These unfavourable traits are mitigated in the body through close proximity of targets to the site of release, sometimes in high concentrations for when multiple targets exist. For peptides to be successful in therapeutic applications, an intense intravenous dosing regimen for the patient is required to maintain an adequate load of the therapeutic. Although hindered by poor bioavailability and short half-life, the biological nature of peptides offers plenty of properties that would make them an ideal therapeutic for complex diseases where specificity and toxicity are of concern [57].

The specific nature of peptides is due to their ability to cover a larger area of the target site compared to SMDs, decreasing the risk of off-target effects that have halted previous clinical trials into AD therapeutics [60]. A benefit of peptides over SMDs is the relative inability to build-up toxicity due to the metabolic instability of the amide bonds that hold the peptide together, resulting in the release of amino acids that can be utilised by various systems [61]. These qualities of peptides are what make them ideal therapeutics for most biological process disorders, specifically those found in the CNS. The delicate environment of the CNS requires therapeutics that are highly specific so as not to affect the normal functioning of the brain, but also produce minimal toxicity to prevent damage to nearby neurons.

3.3 Peptides approved for therapeutic use

In the 36 months that spanned 2016–2018, 8 peptide therapeutics were approved by the FDA making up just over 6% of the drugs approved in that time [62]. This can be looked at in both an optimistic and pessimistic view. However, looking at cumulative FDA approvals given since 1980, there is no denying that peptides have a place in therapeutic development. In 2018, there were over 70 peptides available for medical use in the United States, Europe and Japan, and more than 150 in clinical studies [63]. The most commonly used therapeutic peptides target biological processes in a similar way that biologics, such as proteins, do, replacing molecules that stimulate PPIs. Crucial hormones that were approved for therapeutic use are vasopressin, oxytocin, insulin, glucagon and corticotropin, all of which were approved last century yet still play a pivotal role in HRTs [63].

Currently approved peptides cover a large range of therapeutic areas, such as oncology, metabolic diseases, haematology, respiratory disorders and gastroenterology. Of the peptides approved by the FDA, only three are approved for CNS indications: corticotropin, approved for use in inflammatory diseases; glatiramer, approved for use in MS; and taltirelin, approved for use in spinocerebellar degeneration. After the discovery of taltirelin in 2000, no other peptides have been approved for CNS indications, even though there have been over 30 new peptides approved for other indications since [63]. This begs the question on whether research has moved away from peptides for CNS indications due to their difficulty passing through the BBB, or whether the technology is only now catching up.

3.4 Future considerations for peptide therapeutics for use in CNS indications

With a variety of unfavourable characteristics, peptides require modification prior to clinical testing. The field of peptide synthesis has improved in the past two decades contributing to a rise in more effective peptide therapeutics available for clinical trials [64]. Many traits of peptides that were initially unfavourable have been resolved with new techniques in peptide synthesis. However, there remains the large issue of bioavailability that is restricting the use of peptides as therapeutics for the CNS. The biological nature of peptides reduces their bioavailability, their size making it difficult to cross membrane barriers and the structure of their bonds increasing the rate of degradation in the gastrointestinal (GI) tract and plasma. Due to these features, most approved peptide therapeutics are parenterally administered, involving either intravenous or subcutaneous injections. Parenteral administration allows for the systemic distribution of a relatively large dose of the peptide providing high concentration of the therapeutic when it reaches its target, without having to cross any membrane barriers. The oral route does not allow for this as the conditions are acidic and tight mucosal barriers exist to protect the body from external threats [29].

Administration directly into the blood stream works for many indications where the target is easily reached through diffusion across capillary walls; however, CNS indications are protected from standard blood flow by the BBB. Peptides targeting the CNS endure this extra barrier that acts as a neuroprotective wall, preventing unwanted molecules from entering the sterile and sensitive environment [65]. Studies in transport of drugs across the BBB have shown that there are multiple ways that can be exploited to deliver drugs to the CNS, specifically using transporter pathways that shuttle hormones such as insulin into the CNS [66]. Delivery of previous therapeutics for AD in clinical trials involved either disruption of the BBB, increasing lipid solubility of the molecules or using pre-existing transport systems, with mouse model studies showing effectiveness of the latter two [67]. An alternative route through the olfactory pathway may provide hope for delivering peptides to the CNS; however, intranasal delivery has demonstrated limited progress in clinical settings. Offering an attractive opportunity to bypass the BBB, intranasal delivery presents similar patterns in degradation to other routes of delivery [68].

Although an issue present in the delivery of peptides to the CNS, transport into the CNS is secondary to proteolytic degradation in terms of bioavailability of peptides, with a large proportion of peptide load being degraded before it can reach the target site. Widely accepted as techniques that decrease degradation is conjugation or the production of peptidomimetics, techniques used in peptide synthesis today. The most common conjugate for increasing bioavailability of a peptide is polyethylene glycol (PEG), a molecule that has shown to help prevent clearance of therapeutics. PEG increases the overall size of peptide therapeutics, making it too large for renal clearance and hindering proteolytic cleavage in plasma [30]. Peptidomimetics are a modified form of the peptide that is biologically similar while containing unnatural amino acids or modified peptide bonds [69]. Through the addition of unnatural amino acids and altered peptide bonds, proteolytic enzymes are incapable of cleaving peptidomimetics due to the unnatural nature of the molecule. The process of screening the effects of multiple modifications to the structure of the peptide has improved with the development of simple screening assays, increasing the output of peptidomimetic therapeutics.

4. Conclusions

As a mechanism, neuroprotection in CNS indications where protein misfolding occurs is detrimental, exacerbating the disease by creating inflammation in the local area that leads to the degeneration of nearby neurons. In the case of AD, neuroinflammation occurs when Aβ plaques are recognised by circulating microglial cells, initiating an immune response and releasing pro-inflammatory molecules that lead to the neurodegeneration that is found in patients with AD. The neuroprotective response of microglia in effect begins the deterioration of the brain, calling for therapeutic intervention to aid in neuroprotection.

Current therapeutics used as therapy for AD are all neuromodulatory, addressing the symptoms related to the disease instead of the underlying mechanism. About 73% of the current cohort of therapeutics in clinical trials for AD is DMTs, indicating the need for a therapeutic that either slows or stops the progression of the disease. DMTs targeting the amyloid cascade are of particular interest from a neuroprotective standpoint due to Aβ plaques initiating neuroinflammation. Targeting inflammation and Aβ plaques and fragments will only slow the progression of the disease requiring a more robust target that can stop disease progression. The role of BACE1 in Aβ generation provides an ideal target for therapeutics although it has proved elusive in the past, with trials into SMD inhibitors for BACE1 being halted due to safety concerns from off-target effects.

To develop an ideal therapeutic for BACE1, a molecule that lies somewhere between SMEs and biologics is required. Peptides offer attractive properties from both classes of therapeutic specifically a relative lack of toxicity and great specificity, both of which are ideal for combating CNS indications. Although peptides are seen as inadequate for use as therapeutics, many approved peptide therapies have shown the ability of peptides to be modified, improving qualities that were lacking initially. With further advancements in the field of peptide synthesis and modifications, the number of peptide therapeutics in clinical trials, not just for AD but other indications, will likely increase. Similarly, the number of approved therapies, offering a promising outlook for diseases where therapeutic needs are currently unmet, is likely to increase.

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