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Prospects of β -Secretase Inhibitors for the Treatment of Alzheimer's Disease

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Abstract

β -Secretase continues to be an attractive drug discovery target for the therapeutic intervention of Alzheimer's disease (AD). This enzyme plays a critical role in the production of neurotoxic β -amyloid (A β) peptides in the brain. Over the years, extensive research efforts have led to the development of many promising classes of inhibitors against this protease. Many small-molecule, peptidomimetic, and nonpeptide β -secretase inhibitors have now overcome the key challenging development hurdles such as selectivity and brain penetration. A number of inhibitors have also shown further promise in reducing brain A β and rescuing cognitive decline in animal models. Recently, several β -secretase inhibitors have entered into preclinical and phase I studies, and at least one of these inhibitors has advanced to phase II/III human trials. The outlook on β -secretase inhibitor drugs for the treatment of AD patients is discussed herein.

Keywords

Alzheimer's disease; BACE1; BACE2; cathepsin D; memapsin 2; protease inhibitors; β -secretase

The cloning and identification of β -secretase (memapsin 2, BACE1) nearly fifteen years ago unveiled a long-awaited new therapeutic target for Alzheimer's disease (AD).^[1] The enthusiasm and anticipation for finding new drugs following the discovery were well founded, as this protease occupies a unique position in the pathogenesis of AD. β -Secretase is the first protease that initiates the amyloid cascade in neurons by cleaving the amyloid precursor protein (APP) to generate a soluble N-terminal fragment and a β -amyloid (A β)-containing membrane-bound C-terminal fragment. In the second step, the C-terminal fragment is cleaved by γ -secretase to release toxic A β peptides of various lengths (Figure 1). Of these, the longer isoform, A β 42, is thought to be the most pathogenic. Excess A β in the brain is generally recognized as a leading factor in the onset and progression of AD.^[2] However, this prevailing amyloid hypothesis has been questioned.^[3,4] Nevertheless, both β - and γ -secretases have become drug design targets against AD, a disease that still lacks an effective treatment.

The search for γ -secretase inhibitor drugs has been pursued for more than two decades, and a number of drug candidates have been brought to clinical trials. The universally high toxicity associated with these compounds is a major obstacle for their clinical application and appears to be mechanism based, stemming from many important physiological functions of this protease.^[5] γ -Secretase is a housekeeping enzyme that removes many membrane-associated protein fragments generated by cell membrane proteases including α - and β -secretases. In addition, γ -secretase is involved in other functions such as the signaling mechanism of the protein Notch.^[6] So far, no compensatory activity for γ -secretase has been found and the toxicity associated with drugs that inhibit its activity may continue to be a major issue. β -Secretase, on the other hand, is devoid of many of the function-based problems related to γ -secretase. Therefore, the cloning of β -secretase brought much excitement and optimism as a drug design target. Nevertheless, β -secretase has its own set of obstacles and concerns.

β -Secretase as a therapeutic target has received ample support from preclinical studies. Deletion of this gene in mice essentially abolished A β production and showed only minor phenotypic abnormalities, suggesting that clinical intervention is a viable option.^[7] Such gene deletion also slowed memory decline in transgenic AD mice.^[8,9] Even partial reduction of the β -secretase gene product in heterozygous transgenic mice showed a significant decrease in A β , suggesting that partial inhibition of β -secretase by drugs may render therapeutic benefits.^[7] This contention was supported by administration of a potent BACE1 inhibitor in young transgenic AD mice, and such experiments showed a decrease in brain plaques and lowered the rate of cognitive decline in aging mice.^[10] Although β -secretase is known to have other substrates besides APP, they do not appear to be major stumbling blocks. For example, impairment of learning and memory in mice with β -secretase gene deletion is relatively mild, so the consequences from partial inhibition created by regulating drug dosage may be acceptable. Although β -secretase plays a role in the formation of the myelin sheath in the central and peripheral nerves, this activity occurs only during the prenatal stage, and is thus unlikely to be a factor for treating AD patients at old age.^[11,12] These favorable points and an acute need for an AD drug have generated an explosion of research activities in β -secretase inhibitor drugs during the last decade. The magnitude of the research effort on BACE1 inhibitors is evident by the more than 250 X-ray crystal structures of inhibitor-bound BACE1 in the RCSB Protein Data Base (PDB) along with a large number of patents related to β -secretase inhibitors.^[13]

The evolution toward clinically effective β -secretase inhibitors has been constrained at least in part due to the stringent requirements of drugs for treating a brain disorder. In addition to good pharmacological properties, such drugs must also penetrate the blood–brain barrier (BBB) and brain tissue. Given the fact that AD treatment in elderly patients will be chronic in nature, collective accumulation of drug toxicity over time would be of significant concern. To keep the toxicity to a minimum, such drugs must also have good selectivity against other human aspartic proteases, which are the most likely group to be affected by β -secretase inhibitors owing to their similarity in catalytic mechanism and specificity. Two human aspartic proteases are particularly critical in selectivity design. β -Site APP clearing enzymes 2 (BACE2, memapsin 1) is the closest homologue of β -secretase, and its active-site binding pattern is very similar to that of β -secretase.^[14,15] This protease is present in the human

brain and other tissues, but is not significantly involved in the generation of A β in vivo. Although BACE2 gene deletion produced little significant changes in phenotype, good selectivity against this protease may nevertheless be prudent, because its physiological roles are poorly understood. Cathepsin D is an aspartic protease ubiquitously present in the lysosomes and endosomes of nearly all human cells.^[16] Its critical role in cellular protein catabolism and the high abundance of this enzyme in the human body make it a potential trap for β -secretase inhibitors that lack good cathepsin D selectivity. It also plays an important role in retinal functions of the eyes. The closeness in specificity of these proteases is reflected in the similarity of their active site binding topology, which has made the development of high selectivity particularly challenging. A good β -secretase inhibitor drug candidate needs to be large enough to bind with high affinity while small differences in the binding site structures of these aspartic proteases can be explored for selectivity. On the other hand, such an inhibitor needs to be small enough to show satisfactory pharmacokinetic properties, good BBB penetration, and decreased susceptibility to P-glycoprotein-mediated efflux to cross the BBB. These opposing requirements make β -secretase inhibitor design very challenging.

Efforts over the past fifteen years have brought several β -secretase inhibitors to clinical trials, but so far the ability to modify human AD progression has not been demonstrated. Early on, the availability of X-ray structural information on BACE1–inhibitor complexes spurred structure-based inhibitor design with transition-state isosteres.^[17,18] Many of these early inhibitor design efforts were also facilitated by the availability of structure–activity relationships of the peptidic inhibitors as well as successful drug-design efforts on renin and HIV-1 protease.^[13,19] In general, these peptidomimetic inhibitors achieved excellent β -secretase inhibitory potency by mimicking substrate binding at the transition state. However, the majority of peptidomimetic inhibitors did not have good pharmacological properties such as oral bioavailability, metabolic stability, and BBB penetration. Only a few peptidomimetic inhibitors based on hydroxyethylamine isosteres showed good brain penetration and in vitro pharmacological properties, but most of these inhibitors showed only marginal selectivity against BACE2 and cathepsin D.

Subsequent research efforts toward nonpeptidic inhibitors/ scaffolds and further modification of peptidomimetic inhibitors led to the evolution of BACE1 inhibitors with drug-like properties, showing in vivo A β reduction in laboratory animals. Also notable is the evolution of small-molecule nonpeptide BACE1 inhibitor classes containing carbinamine, acyl guanidine, aminothiazines, and aminoquinazoline-based scaffolds. These compounds inhibit BACE1 by interacting with the catalytic aspartates as well as with the specificity pockets of BACE1. A number of inhibitors exhibited good potency, pharmacokinetic properties, and BBB penetration in animal models. Recent high-throughput and fragment-based screening campaigns identified a broad range of promising lead structures that show good potential to overcome ADME hurdles. However, the majority of this class of inhibitors lack selectivity against BACE2 and cathepsin D.^[13,19]

Overall, steady progress has been made in the structural evolution of BACE1 inhibitors with desirable drug-like properties. A number of potent and relatively small inhibitors have been shown to cross the BBB and reach the brain. So far, several BACE1 inhibitors are reported to

have completed phase I clinical trials, and one inhibitor has entered into phase II/III clinical development. It may take years before convincing results can be generated; in the meantime, however, some relevant information has been obtained from a BACE1 inhibitor (GRL-8234) study in transgenic AD mice over a seven-month period.^[10] The results from these studies shed some light on the setting of human clinical trials with AD patients. While transgenic AD mice do not truly replicate human AD, these mouse models do share some early features of human AD. These include the production of excess A β in the brain, a decline in cognitive function with age, and the development of amyloid plaques in the brain at middle to late age. The administered drug, GRL-8234, is a potent and moderately selective (against BACE2 and cathepsin D) BACE1 inhibitor. The drug was shown to rescue age-related cognitive decline and was found to decrease brain amyloid levels in Tg2576 transgenic AD mice. The studies indicate that even a partial decrease in A β production and brain plaque load of ~50 % is sufficient to rescue cognitive decline. There was no observable toxicity even though drug treatment was carried out for a period of seven months. Notably, APP accumulation in the brain was not significant, although this mechanism-based side effect was of concern. This result suggested that an alternative APP processing pathway by α -secretase may have been operative. It was quite surprising that it took several months for drug-treated mice to manifest cognitive rescue. A further interesting observation was that BACE1-inhibitor-treated old mice with brain plaques failed to show cognitive rescue. While these results are very informative and may not duplicate in human clinical trials, the studies raised some important points. In particular, clinical trials with early stage AD patients over a longer trial period may manifest clinical benefit over patients with late-stage AD.

Extensive medicinal chemistry efforts have provided a number of promising classes of BACE1 inhibitors for clinical development.^[20,21] CTS-21166 (CoMentis) was the first publicly announced BACE1 inhibitor that underwent a phase I clinical trial in 2007.^[22] This inhibitor showed good potency (BACE1 K_i = 2.5 nM, cellular potency IC_{50} = 3 nM) and selectivity (BACE2 and cathepsin D selectivity: ~100-fold). It was administered in 13-month-old transgenic AD mice for six weeks at 4 mg kg⁻¹ and resulted in a decrease in brain A β by about one-third. Chronic dosing of this inhibitor resulted in no detectable change in the myelination of peripheral nerves in the mice. Phase I clinical trials of this compound were conducted in healthy young males to evaluate safety and preliminary A β responses. The drug was infused, and a dose-dependent decrease in plasma A β was reported. Plasma A β levels reached ~80 % inhibition for the highest dosage at 4–8 h. Similar results were observed if 200 mg of CTS-21166 was administered in an oral liquid solution.^[22,23]

A small-molecule nonpeptidic BACE1 inhibitor, LY2811376 (Eli Lilly), was evaluated for safety and pharmacological properties in animal models as a prelude to phase I clinical trials.^[24] This compound was reported to have good ADME properties (BACE1 IC_{50} = 240 nM, cellular potency IC_{50} = 300 nM) and selectivity (BACE2 and cathepsin D selectivity: ~10- and 65-fold, respectively). However, the compound showed non-target-associated retinal toxicity in rats, and its development was discontinued. A next-generation compound, LY2886721 (Eli Lilly), was then advanced to clinical development. In preclinical animal studies, the drug did not show any retinal or brain toxicity.^[25] The drug was administered orally for 14 days in phase I clinical studies. It was reported to be safe and well tolerated, showed a plasma half-life of ~12 h, suitable for once daily dosing. The compound advanced

to phase II clinical trials for six months with mild AD patients. However, the phase II trial was terminated due to abnormal liver biochemical results. It was reported that liver abnormalities were not related to the BACE1 mechanism. However, this view has been debated recently.^[26]

MK-8931 (Merck) is a small-molecule BACE1 inhibitor that is in phase II/III clinical development.^[27] In a phase I trial, the drug was reported to be safe and decreased cerebrospinal fluid (CSF) A β concentrations in AD patients. In subsequent phase I/ II trials, the drug was reported to decrease CSF A β by up to 90 percent. This compound has now entered into phase II/III development with patients of mild to moderate AD.^[28] A Phase III clinical study with AD patients with mild cognitive impairment has also started. Another new BACE1 inhibitor, AZD3293 (Astra-Zeneca), recently entered into clinical development.^[29] This compound was evaluated for safety and tolerability in elderly AD patients; the compound was reported to be safe and showed significant reduction of CSF A β levels. Based on these results, AZD3293 has advanced to phase II/III clinical development by Eli Lilly and AstraZeneca. A number of other BACE1 inhibitors have been reported to enter into clinical development. A small-molecule BACE1 inhibitor, E2609 (Eisai), has completed phase I studies.^[30] It was recently reported to have entered into phase II development in a joint partnership with Biogen Idec. Additionally, there are at least three other BACE1 drug candidates (RG-7129, HPP-854, AZD3839) that have entered into clinical development.^[19]

It has been fifteen years since β -secretase was cloned and the first X-ray crystal structure of inhibitor-bound BACE1 was determined. Although a number of β -secretase inhibitor drugs are now in advanced clinical development, the progression of such endeavors has been very challenging. However, structure-based design cycles and intensive medicinal chemistry efforts are starting to pay off. There was a flurry of early clinical results on β -secretase human trials which raised significant renewed attention on this target. Therefore, what have we learned so far from these clinical trials? The most convincing result is that β -secretase inhibitor drug treatment indeed significantly reduces A β in the brain, and one drug has been shown to cause a 90 % decrease in CSF A β levels. The available sporadic results suggest that, for the most part, a number of β -secretase inhibitor drugs are well tolerated. However, there are β -secretase inhibitors that have been terminated due to compound-related toxicity and not related to β -secretase mechanism. These results further underscore the importance of inhibitor selectivity against physiologically important proteases. It may be that an increased selectivity of 1000-fold or better over BACE2 and cathepsin D may be necessary to achieve the desired results.

It is quite exciting that the next phase II/III trials may provide a response to the most daunting question of drug efficacy and the effective stage of AD at which a BACE1 drug will be likely to show optimum results. These new phase II/III trials will continue to be very challenging, as they are entering into uncharted territory. Without an approved biomarker for AD clinical trials, the efficacy test must rely on statistical significance on the difference of cognitive performance over a length of time. The slow nature of cognitive decline in AD may translate into a need for extraordinarily long trials. Another important point is then which patient group may show the best observable difference. As suggested during GRL-8234-based cognitive studies in transgenic mice, early stage AD patients may show a

benefit from β -secretase treatment over late-stage patients in which secondary pathology such as inflammation may complicate an A β reduction benefit. The overall progress in medicinal chemistry of β -secretase inhibitors has been very impressive. The enormous amount of effort in this field has now brought us to a critical juncture, where a number of structurally diverse β -secretase inhibitors are in advanced clinical trials. The long-awaited question of therapeutic efficacy of β -secretase inhibitor drug treatment will likely be answered by these works. It seems probable that β -secretase will fulfill its promise as a disease-modifying target and a safe and effective treatment for AD patients.

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Figure 1.
APP processing pathways by proteases.