Chapter 1

Amyloid Beta Hypothesis: Attention to β- and γ-Secretase Modulators

Jan Korabecny, Katarina Spilovska, Ondrej Soukup, Rafael Dolezal and Kamil Kuca

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75629

Abstract

The amyloid cascade hypothesis poses one possible explanation for the onset and progression of Alzheimer's disease (AD). With this respect, neurotoxic effect is attributed to soluble and diffusive amyloid-β (Aβ) oligomers. Aβ peptides are produced by proteolytic cleavage of the hydrophobic transmembrane portion of the amyloid precursor protein (APP) by successive action of β- and γ-secretases. Aβ peptides are generated in several isoforms, out of which the most pronounced are Aβ40 and Aβ42 being the major constituents of amyloid plaques found in AD patients' brains. Since the indisputable evidence pointed out to Aβ oligomers as toxic agents, several pathways to modulate or control the aggregation have been inspected. Given all these aspects, inhibitors of the β- and γ-secretases have gained the most attention. This chapter presents amyloid cascade hypothesis with current progress in the development of β- and γ-secretase modulators to counteract the Aβ burden.

Keywords: Alzheimer's disease, amyloid beta, neurodegeneration, amyloid precursor protein, β-secretase, γ-secretase, presenilin

1. Introduction

Despite the great progress in understanding pathogenetic and pathological processes associated with Alzheimer's disease (AD) in the last decade, the exact cause of AD still remains unrevealed. With the aim to clarify this cause, a number of hypotheses have been proposed, which involve, for example, the genetic hypothesis of AD based on malfunctioning variants of apolipoprotein E genes (*APOE*), the hyperphosphorylation of cytoskeletal proteins (especially of tau protein) or the theory of oxidative stress [1]. Importantly, AD is often explained by inflammatory processes in the brain, and metabolic processes leading to the formation and accumulation of the

beta-amyloid (Aβ) [2]. Among all these theories, the amyloid metabolic cascade or the amyloid hypothesis and posttranslational modification of tau protein are considered as the main pathophysiological theories elucidating the outbreak of AD, although none of them is able to sufficiently explain the diversity of the biochemical and pathological abnormalities associated with the developed AD [3].

According to the amyloid hypothesis, slow accumulation of extracellular senile plaques, composed of Aβ deposits, occurs in the beginning and further progresses into AD. On the other hand, a direct link between the toxic influence of Aβ, the impaired neuronal functions and the decline in memory functions still has not been fully clarified, but it is broadly accepted that Aβ undoubtedly plays a key role in the neuropathology of AD [4].

2. Amyloid precursor protein

Amyloid precursor protein (APP) is an integral membrane glycoprotein that is expressed in the brain and the central nervous system (CNS). APP can be cleaved by specific proteases in two different pathways: α-path and β-path [5]. In most cases, APP is cleaved in the α-path with the participation of enzymes α- and γ-secretases. The cleavage of APP by α-secretase proceeds in the way, which can be described as non-amyloidogenic one, while the cleavage in the β-way leads to formation of the toxic fragments of Aβ. In the case the non-amyloidogenic path, APP

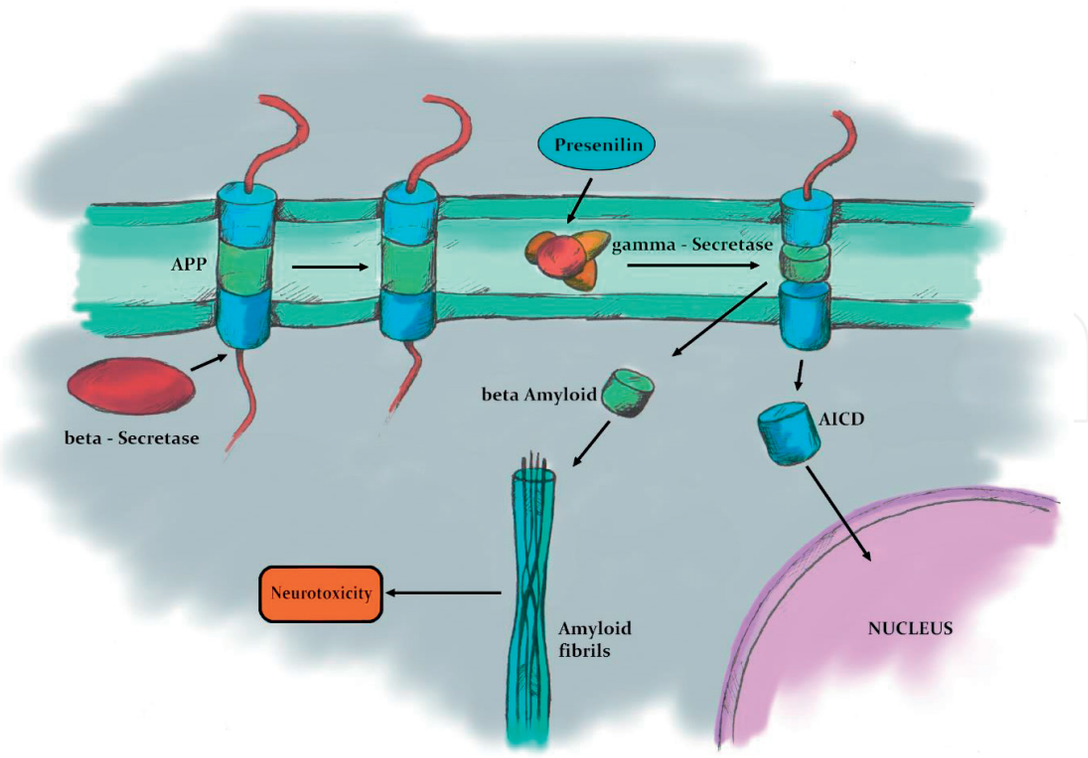

Figure 1. Scheme of the amyloidogenic processing of APP.

is cleaved by α-secretase to form a soluble extracellular fragment of sAPP-α and C83 fragment, which is split by γ-secretase, similar to the case of C99 [6]. However, the α-path does not release Aβ, but it leads to splitting out a short protein fragment p3. The exact physiological function of the fragment p3 has not been completely clarified yet [7]. In the course of the β-path, APP is first cleaved by the enzyme β-secretase (BACE-1) providing the *C*-terminal fragment of the length of 99 amino acids (C99) and a chain, which is transferred to the extracellular space. This remaining protein chain can be found in the literature under the acronym sAPP-β. Subsequently, C99 is cleaved by the activity of γ-secretase to short-length peptides consisting of 38–43 amino acids (referred to as Aβ) and the intracellular *C*-terminal domain (AICD). In most cases, formation of $A\beta_{1–40}$ mainly occurs, although a longer and more toxic form $A\beta_{1–42}$ sometimes can be also produced. However, recent findings also point to the fact that the production of Aβ can take place even within the proteolytic cleavage of APP along the α-path (**Figure 1**) [8].

3. Physiological function of amyloid precursor protein

Although APP is a part of the pathophysiological processes involved in AD, it is clear that the protein also carries out several natural physiological functions, particularly within the regulation of the synaptic transmission. It has been proved that transgenic mice with knock-out gene for APP exhibited an inability to transmit signals to the neuromuscular junction. Despite this fact, mice with upregulated expression of APP show better cognitive functions and spatial orientation. This is often rationalized by overproduction of AICD given by γ-secretase. The activity of APP is also put in a close connection with the metabolism of cholesterol. The neuroprotective character of APP was also demonstrated by suppression of the cyclin-dependent kinase 5 (CDK-5) activity in the process of tau hyperhosphorylation [9].

4. Pathological features of amyloid precursor protein

The pathological role of APP is generally associated with the amyloidogenic way of its splitting. In general, many mutations of APP cause the autosomal dominant form of AD with early onset. Interestingly, genetic mutations in the adjacent part of the β-site of the APP gene induce neuroprotective effects, because Aβ is then produced only in a small extent. On the other hand, an excessive expression of the mutated APP forms associated with FAD (a redox cofactor in a number of biochemical reactions) leads to a loss of sense of smell, without dissemination of amyloid plaques, though. This observation is in a line with the loss of sense of smell, which occurs in some patients in the early stages of AD [9].

5. β-secretase

β-secretase (BACE-1; also referred to as Asp2 or memapsin 2) is an enzyme that breaks down APP in the site called β into the *C*-terminal fragment, from which monomers of Aβ are

subsequently formed in the neurons. BACE-1 and the homologous BACE-2 are regulated differently and also control different processes. A disrupted intracellular calcium homeostasis may stimulate the genetic expression of BACE-1 *via* triggering the nuclear factor of activated T-cells of type 1 (NFAT1), which leads to over-production of Aβ. Expression of the BACE-1 can also be controlled by the level of $A\beta_{1-42}$ (but not by the $A\beta_{1-40}$) through some transcription factors. In addition, some plaques containing $A\beta_{1-42}$ even increase the levels of BACE-1 in the adjacent neurons just before their death [10]. The homologous enzyme BACE-2 shares 64% of the sequence identity with BACE-1. The action of BACE-2 is in many aspects similar to the activity of α-secretase. BACE-2 triggers a cascade of cleavage of APP by the non-amyloidogenic way. Its physiological function is associated with the organ pigmentation [11].

In order to clearly demonstrate the involvement of BACE-1 in the pathogenesis of AD, many prominent scientific groups worldwide dealt with developing a mouse model that had deactivated the gene for the production of BACE-1 (i.e., BACE-1 knockout (−/−) mice). At first, these strains of mice were viable, capable of reproduction, with the normal morphology of the body, without any obvious signs of damage of the tissues and normal blood picture [12]. This finding supported the idea that inhibition of BACE-1 can bring about the desired therapeutic effect without adverse effects. The results of this study also point to the fact that the related BACE-2 fails to offset the activity of BACE-1 in the formation of Aβ. It is interesting that hybridization of these BACE-1 knockout (−/−) mice with transgenic mice having the APP gene, which increasingly produce amyloid plaques, provided a generation, the newly born individuals of which did not exhibit the formation of Aβ, Aβ deposits or signs of memory impairment caused by production/accumulation of Aβ. As already mentioned, BACE-1 is located mostly in the presynaptic endings of neurons, where its physiological effects is assumed to occur. Over time, however, it was found that BACE-1 knockout (−/−) mice had impaired axonal conduction, experiencing hypomyelinization (i.e., disrupted formation of myelin, the substance that surrounds the axons and nerve fibers), memory disorders, disturbed neurochemical balance, pathological neurogenesis, astrogenesis, degeneration of neurons with increasing age, pathological changes in the retina and schizophrenic symptoms. All these discoveries observed in BACE-1 knockout (−/−) mice can serve as a model that reflects the potential adverse effects associated with the administration of BACE-1 inhibitors for normal animals or people [13].

The substrates subject to proteolysis by BACE-1 are in particular the membrane-bound proteins like APP. Many of these BACE-1 substrates undergo a process called ectodomain shedding (ectodomain is a part of a membrane protein which protrudes to the extracellular space), while at the same time, these substrates can be cleaved by proteases, called also disintegrins, and ADAM-related metaloproteases. The extent of cleavage of the substrate by ADAM related proteases or BACE-1 depends on the nature of the particular substrate. All the possible side effects caused by inhibition of BACE-1 thus may not be always exhibited, assuming that some substrates are hydrolyzed by another protease [14].

The homology between BACE-1 and BACE-2 gave rise to arguments that BACE-1 inhibitors may simultaneously inhibit non-selectively also BACE-2. For this reason, transgenic BACE-2 knockout (−/−) mice were developed to clarify the physiological role of BACE-2 and to explore the benefits offered by inhibition of this enzyme. Similar to the BACE-1 knockout (−/−) mice, the

BACE-2 knockout (−/−) mice showed the same phenotype. Double-knockout mice, that is, mice with deactivated genes for BACE-1 (−/−) as well as for BACE-2 (−/−), are not phenotypically very different from mice without the gene for the BACE-1, with the exception of an increased number of dying mice freshly after birth. The results of this study therefore assume that nonselective inhibitors of both subtypes of the enzyme BACE may be well tolerated at least from the perspective of the inhibition of BACE-2. The latest research has shown that BACE-2 is expressed in the pancreatic β cells and BACE-2 knockout mice exhibit an improved glycemic regulation due to the increased production of insulin. These findings imply the possible use of BACE-2 inhibitors for the treatment of diabetes mellitus of type 2 [15].

6. BACE-1 inhibitors in the treatment of Alzheimer's disease

Currently, BACE-1 inhibitors have an exclusive position regarding the therapeutic options for introduction into clinical practice to treat AD [16]. Their mechanism of action is based on reducing the levels of Aβ in the brain. Although several of these inhibitors had already reached clinical testing, there are still important questions to answer, for instance, about their safety, the optimum degree of inhibition of BACE-1 needed to achieve the desired therapeutic effect without the presence of side effects, and the stage of the disease when these compounds are to be indicated in order to achieve the greatest assets [17].

Aβ is produced by neurons in the brain, partly also by astrocytes and other glial cells, which are involved in the formation of this protein in particular during the stress conditions accompanying the AD development. For the production of Aβ, the activity of both enzymes, BACE-1 and γ-secretase, is necessary [10]. The biochemical processes involving the activity of these enzymes are often referred in the literature as the amyloid pathway. Importantly, modulation or inhibition of these enzymes can reduce the formation of Aβ in the brain of patients with AD. On the other hand, activation of the non-amyloidogenic pathway by supporting the α-secretase activity may also reduce the formation of Aβ and currently it is alternatively considered as a promising approach for therapy of AD. An important role for the accumulation of Aβ is also played by the genetic aspects of AD. Nowadays, more than 200 autosomal-dominant mutations in APP and presenilin (PS) have been identified which contribute to the occurrence of familial forms of AD [18]. Without any exception, all these mutations increase the production of all Aβ isoforms, in particular the toxic Aβ containing 42 amino acids ($A\beta_{1–42}$). An example might be seen in Swedish mutation of APP in the amino acids Lys670 and Met671, that is, the places where BACE-1 enzyme cleaves APP. This mutation results in higher proteolytic efficacy of BACE-1, which promotes an increased rate of the C99 fragment formation and thereby the total production of Aβ [19]. The *APOE-ε4* allele represents a major genetic risk factor for the development of AD with the late onset and it is also associated with an increased production and accumulation of Aβ. Similarly, mutation of ADAM10 metaloprotein, which is endowed with physiologically similar activity to that of α-secretase in neurons, causes the late onset of the AD by suppressing this enzyme activity, while the amyloidogenic cleavage of APP by BACE-1 prevails [20]. Recently, at least five different genes whose mutation contributes significantly to the increased formation of Aβ have been identified. Based on all

these mutations and their effects, we can conclude that Aβ is responsible for the pathogenesis leading to the breakout and development of AD. Accordingly, some mutations in APP can represent a protecting mean to suppress the progression of AD. For example, a mutation in the Ala673 region (so-called Ala673Thr mutation) causes a lower affinity of APP to BACE-1, bringing the production of Aβ reduced by up to 40% [21].

Extensive research is dedicated to the development of small molecule inhibitors of BACE-1, capable to act centrally. The first experimental inhibitors were derived from short fragments of APP, being therefore peptide derivatives. These differed from APP by modified amino acid sequence and increased metabolic resistance against cleaving by BACE-1. In *in vitro* conditions these bulky peptide derivatives showed high affinity for BACE-1, especially due to the fact that the active site of BACE-1 is so large that it is able to cleave very large substrates. The disadvantage of these derivatives is that they do not possess true drug-like properties, exerting low oral availability and short half-life in the plasma. Such drug candidates are quickly metabolized and have low permeability through the blood-brain barrier. For these reasons, the researchers have focused on the development of small BACE-1 inhibitors that have high affinity for this enzyme, but are small enough to penetrate through the blood-brain barrier and, at the same time, to exhibit suitable pharmacokinetic properties. In addition, these compounds must be lipophilic enough to permeate through the cytoplasmic and endosome membrane to block the active site of BACE-1 located inside of the lumen. A large number of these compounds, however, reached only a limited concentration in the brain because in most cases, they reached high efflux mediated by P-glycoprotein (P-glycoprotein is an ATP-dependent pump, which removes xenobiotics and protects the brain from the effects of these compounds) [22].

The latest generations of BACE-1 inhibitors are characterized by a good capacity to permeate through the blood-brain barrier, by a suitable pharmacokinetic profile, and the ability to induce reduction of the cerebral levels of Aβ. The result of the research is a panel of several inhibitors of BACE-1, which have entered various stages of clinical testing [23].

6.1. MK-8931

In 2012, the results of the first phase of clinical trials with inhibitor MK-8931 were presented (**Figure 2**), which had been performed in 88 healthy individuals aged between 18 and 45 years. The safety, tolerability, pharmacokinetic and pharmacodynamic parameters after single or repeated administration were experimentally determined. MK-8931 was generally well tolerated, and no severe side effects were observed. The main goal in this first phase was to determine whether MK-8931 was capable of penetration into the brain to inhibit the activity of BACE-1. Biomarkers monitoring the levels of $A\beta_{1-40}$, $A\beta_{1-42}$ and soluble fragment of APP (sAPP-β), which is formed by BACE-1, were intensively studied. MK-8931 significantly decreased the concentrations of cerebrospinal Aβ, depending on the dose administered, and even in repeated oral administration a reduction of Aβ in the CSF of up to about 90% has been observed. The plasma half-life of MK-8931 after a single administration was around 20 h, which assumes the dosing schedule within the range of a single daily dose. This was followed by a clinical study 1b, where the safety, tolerability, pharmacokinetics and pharmacodynamics in 32 patients with mild to moderate dementia of the AD type were determined.

http://dx.doi.org/10.5772/intechopen.75629

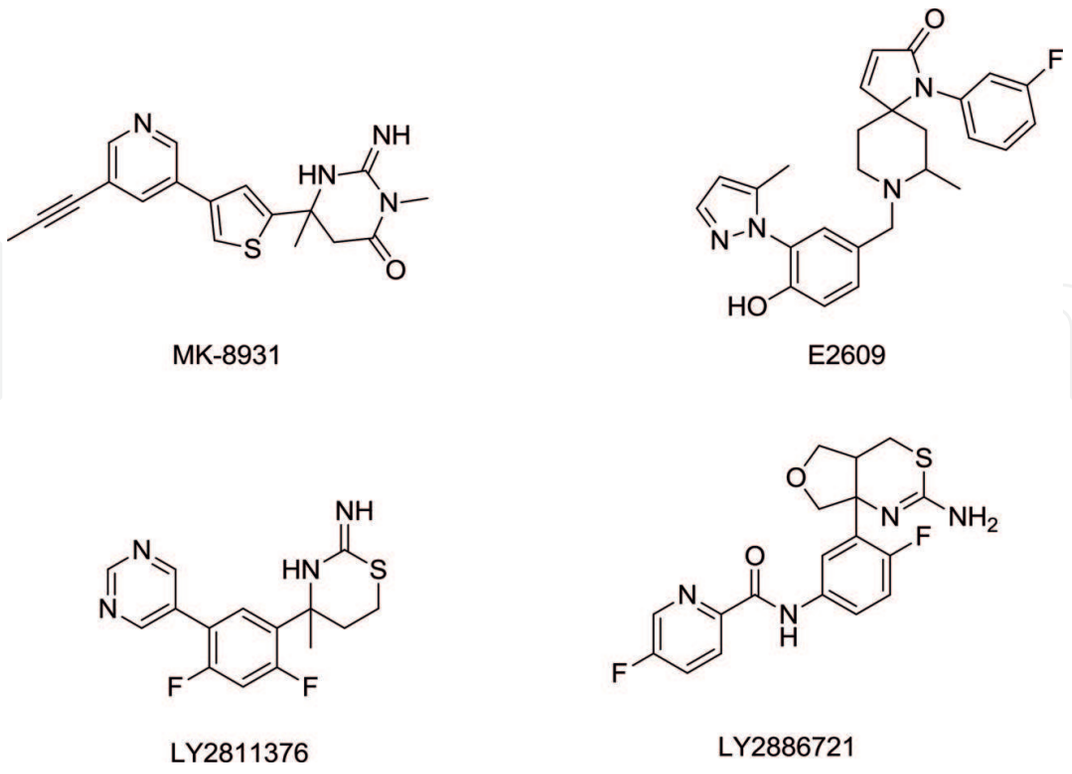

Figure 2. Chemical structures of BACE-1 inhibitors in clinical testing.

MK-8931 was applied in three different doses (12, 40 or 60 mg) and the effect was compared with the placebo over a period of 7 days. The markers of $A\beta_{1–40}$, $A\beta_{1–42}$ and sAPP-β were also monitored. As in the previous phase, decrease in the levels of Aβ, depending on the dose of the drug (for the $A\beta_{1–40}$ 57% (12 mg), 79% (40 mg), 84% (60 mg)) was observed and, in addition, without the presence of the more serious side effects. The results of this phase of clinical trials are especially important because the pharmacokinetic and pharmacodynamic properties of this BACE-1 inhibitor are not affected by the quantity of Aβ present in the brain of patients with AD. At the end of 2012, MK-8931 advanced to the clinical phase (II/III) with patients suffering from mild to moderate dementia of AD type. This substance was administered in dosages of 12, 40 and 60 mg and controlled with placebo in the total sample of 200 patients. According to the initial promising results, extension of the third phase of clinical trials by another 1960 patients with AD is expected. Further evaluation of MK-8931 is simultaneously monitored within the III phase of clinical testing on 1500 patients with AD. The results of both studies are to be expected in 2017–2018 [24].

6.2. LY2886721

A non-peptidic BACE-1 inhibitor LY2811376 (**Figure 2**), which was analyzed in a study with oral administration, demonstrated satisfactory pharmacokinetic and pharmacodynamic properties in animal models, which promoted the compound to the first phase of clinical trials. These clinical studies, however, were soon discontinued due to adverse reactions, in particular in the area of inflammation of the retina and the occurrence of stroke. Although all other studies

with the substance faded away, at present, LY2811376 has become a lead structure, which could be administered orally and reach its biological target behind the blood-brain barrier.

The molecule marked with LY2886721 (**Figure 2**) represents the next evolutionary generation of orally acting BACE-1 inhibitors, which has entered into the second phase of clinical trials. Compared to its predecessor LY2811376, the novel drug LY2886721 did not exhibit any side effects in the area of the retina and any stroke. During the first phase of clinical trials on 47 healthy volunteers, no adverse effects were observed in 14 days (different dosing schemes—repeated administration of 5, 15 and 35 or 70 mg single administration). The biological half-life fluctuated around 12 h, allowing the dosing once per day, when the drug holds the necessary biological effect even after substantial elimination from the body. Treatment with LY2886721 resulted in the reduction of the plasma and cerebrospinal levels of $A\beta_{1-40}$ by up to 74% (i.e., after the highest dose of 70 mg). Similar decreasing changes were detected in the cerebrospinal levels of $A\beta_{1-42}$ and sAPP-β, while the blood level of sAPP-α was increased, which is logically explainable by relative excess of α-secretase in comparison with BACE-1.

The second phase of clinical trials with LY2886721 was carried out in 130 patients with moderate to severe AD dementia type. This testing, however, was terminated because of liver abnormalities, but, presumably, this is not associated with inhibition of BACE-1 [25].

6.3. E2609

E2609 (**Figure 2**) is an orally available, nonpeptidic spirocyclic inhibitor of BACE-1, which induced a significant decline of brain levels of Aβ in preclinical studies. Based on this success, E2609 entered the first phase of clinical testing in which 73 volunteers, administered uniformly with increasing dose from 5 to 800 mg of the drug, and 50 volunteers, administered with different doses in the range of 25–400 mg, participated. The plasma half-life of E2609 is around 12–16 h, which again allows one-day dosing schedule. At the maximal single dose (400 mg), decrease of the cerebrospinal Aβ levels by up to 85% has been observed. The concentration of sAPP-β has been similarly reduced, while sAPP-α has been increased. Currently, the drug is in the third phase of clinical determinations [26].

7. γ-Secretase

γ-Secretase is a member of aspartic protease family that cleaves glycoproteins of type I including APP. Unlike β-secretase, γ-secretase has a regulated intramembrane proteolytic activity (RIP), thus, it breaks down domains inside of the cytoplasmic membrane. It is known that it breaks down multiple substrates, and to this day more than 50 such substrates, including APP, have been identified. Among these substrates are Notch, Jagged and Nectin-1α. The signal transmission by RIP is implemented so that the released intracellular domain is moved into the nucleus, as it is in the case of Notch, which regulates specific gene expression. Notch is therefore cleaved to Notch intracellular domain, NICD, which causes in the nucleus the mentioned regulation. In relation to AD, this signal pathway is interesting from the perspective of development and function of the nervous tissue.

Over the last few years, it has turned out that four main factors are responsible for the enzymatic activity of γ-secretase complex: presenilin, anterior pharynx-defective, presenilin enhancer 2 and nicastrin, which are described further in this chapter [27].

8. Inhibitors of γ-secretase in the clinical development

In recent years, a series of potential inhibitors of γ-secretase has been designed and synthesized. Unfortunately, most of them are not specific to cleaving APP with γ-secretase, and, like in the case of BACE-1, they prevent processing of other γ-secretase substrates that do not have any or at least no obvious role in the pathogenesis of AD. For these reasons, the inhibition of γ-secretase has been associated with serious side effects, which adumbrated the end for most drug candidates in clinical testing.

Historically, the first inhibitor of γ-secretase that underwent clinical studies was BMS-299897 (**Figure 3**) compound prepared by Bristol-Myers Squibb. In 2001, clinical trials of this molecule

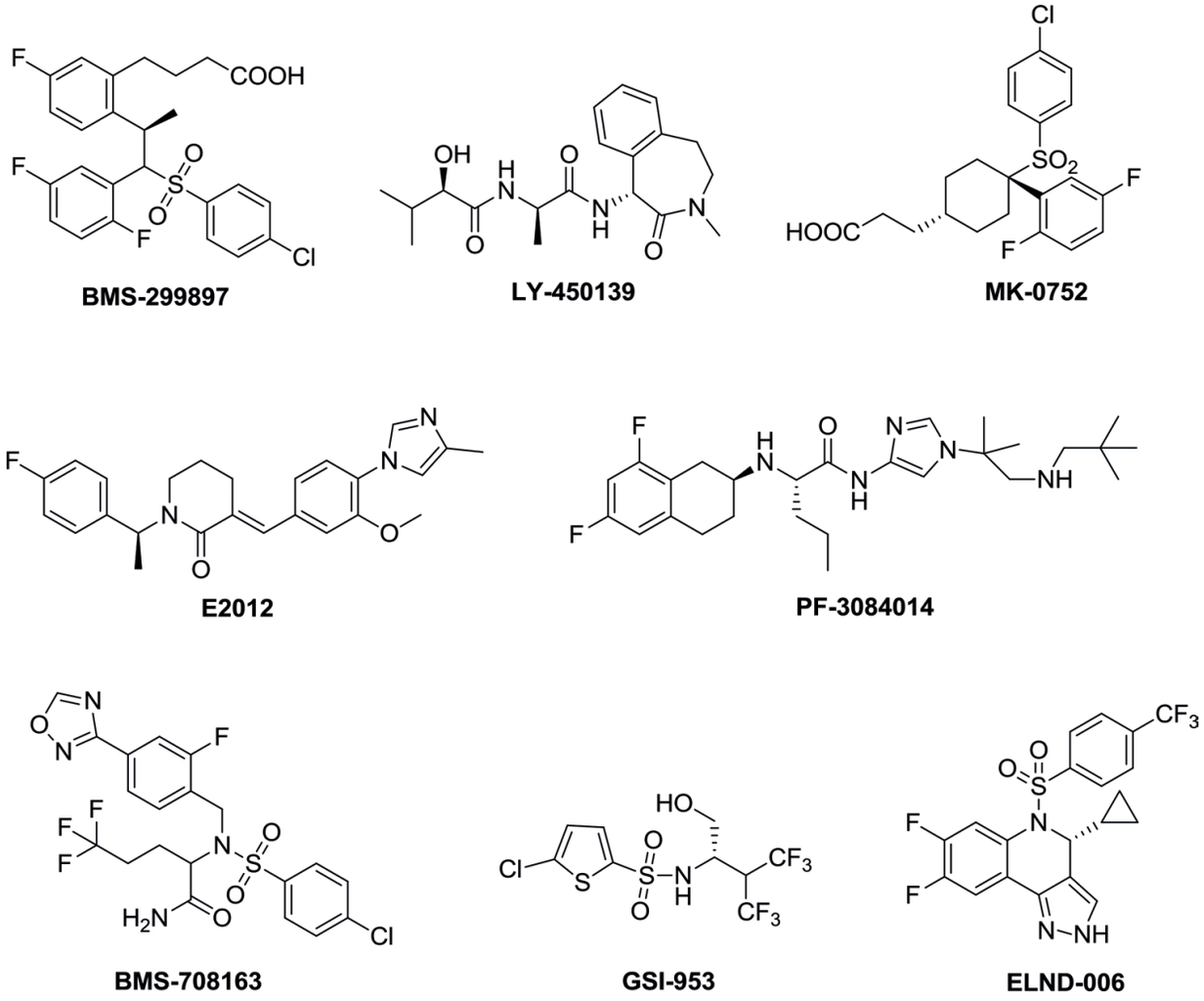

Figure 3. Inhibitors of γ-secretase in various stages of clinical testing.

began, but the results of this study have never been fully described. We only know that the next clinical trials have been terminated.

Six other inhibitors of γ-secretase are currently in various stages of clinical testing involving patients suffering from AD. As for the compounds LY-450139, MK-0752, BMS-708163, PF-3084014, GSI-953 and ELND-006, animal studies indicated that these substances reduced the brain levels of Aβ after oral or parenteral administration (**Figure 3**) [28].

8.1. LY-450139

LY-450139 (**Figure 3**), also known as semagacestat, is an inhibitor of γ-secretase developed by Eli-Lilly. It is a derivative of benzoazepinone with triple selectivity to inhibit the cleavage of APP with respect to the cleavage of Notch (APP: IC_{50} = 15 nM and Notch: EC_{50} = 49 nM). This derivative has undergone all three phases of clinical trials. When tested on experimental animals, it was found that LY-450139 has an effect on the level of Aβ in the brain, cerebrospinal fluid (CSF) and the plasma in mice, guinea pigs and dogs. A similar positive effect was achieved in the cerebrospinal fluid (CSF) of AD sufferers. However, due to the neurotoxicity detected in transgenic mice, gastrointestinal problems and an increased risk of developing skin cancer in humans, clinical testing was abandoned [29, 30].

8.2. MK-0752

This substance developed by Merck is a non-selective inhibitor of APP and Notch formation. In healthy volunteers, MK-0752 (**Figure 3**) administration led to reduction of $Aβ_{1–40}$ levels in the CSF. However, the drawback was the mentioned non-selectivity toward Notch cleavage and significant toxicity in humans. MK-0752 has reached only the first phase of clinical testing [31].

8.3. E2012

The drug E2012 (**Figure 3**) was developed by Eisai in cooperation with Torrey-Pines Therapeutics with the aim to reduce the levels of Aβ by modulating the γ-secretase without affecting the Notch. In mid-2006, the first phase of clinical testing has started, but in February 2007 it has been suspended due to the lenticular opacity observed in preclinical studies with rats. In the time of the study suspension, however, no health problems in humans were observed. In addition, the lenticular opacity has not appeared in later studies in monkeys. During a subsequent study, no eye toxicity was observed in rats, and, thus, the suspension of testing was repealed in April 2008. Currently, the drug is no longer the subject of research interests, anyway [32].

8.4. BMS-708163

The drug identified as BMS-708163 (**Figure 3**) is a benzene sulfonamide developed by Bristol-Myers Squibb. This molecule exhibits nearly 200× lower selectivity to Notch cleavage ($Aβ_{1–40}$: IC_{50} = 0.3 nM and Notch: EC_{50} = 58 nM). Animal studies, specifically in rats and dogs, have shown the ability of BMS-708163 to reduce the levels of Aβ in the brain and the CSF without the Notch-related gastrointestinal and lymphoid toxicity. Despite the fact that reduction of the Aβ level in the CSF has been observed in healthy volunteers, there is insufficient information on storing Aβ

plaques in the brain of transgenic mice, as well as on their behavioral changes. This inhibitor has passed the phase II of clinical development, but further testing is currently not being performed [33].

8.5. PF-3084014

PF-3084014 (**Figure 3**) is a new effective, aminotetraline based γ-secretase inhibitor developed by Pfizer, which does not affect Notch. In in vitro tests, the compound was evaluated as an uncompetitive and reversible inhibitor of human γ-secretase with IC_{50} = 6.2 nM. In studies on tissue cultures, it seems as a weak inhibitor of Notch with IC_{50} = 1915 nM. The ratio between the APP and Notch selectivity is roughly 1500. The merit of this compound is a sufficient penetration through the blood-brain barrier, long-term effect on reducing the Aβ levels and no rebound phenomenon for levels of Aβ in animal plasma. As in the case of BMS-708163, there is also a lack of data for this inhibitor about the storage of Aβ plaques in transgenic mice, as well as about their behavioral processes. PF-3084014 is currently introduced into the second phase of clinical testing [34].

8.6. GSI-953

This inhibitor, known also as begacestat, is a potent thiophene-related sulfonamide developed by Weyth. It is able to suppress the production of Aβ in low nanomolar concentrations in vitro (IC_{50} = 8 nM) and in vivo ($Aβ_{1–42}$: IC_{50} = 15 nM). Cellular studies on the Notch cleavage showed 15× higher selectivity of this molecule to inhibit preferably the cleavage of APP. It was found that GSI-953 (**Figure 3**) improves the memory functions in transgenic mice; however, it does not diminish the level of $Aβ_{1-40}$ in the CSF in people suffering from AD. This drug completed the first phase of the clinical trials, but the lack of its efficacy caused it to no longer be a subject of follow-up studies [35].

8.7. ELND-006

The inhibitor ELND-006 (**Figure 3**) developed by Elan Pharmaceuticals shows increased selectivity for inhibition of the APP cleavage (IC_{50} = 0.34 nM) with regard to Notch cleavage (IC_{50} = 5.3 nM). Therefore, it does not significantly affect Notch; it has a good penetration through the blood-brain barrier and can reduce the level of Aβ in the brain in transgenic mice. The disadvantage of this drug is the rebound phenomenon in the plasma of animals and lack of data on behavioral processes in animal models of AD. Clinical studies of the drug have been terminated because of severe hepatic adverse reactions, which presumably are not related to the mechanism of γ-secretase inhibition by the drug [36].

9. Presenilins 1 and 2

Presenilins (PSs) are membrane proteins encoded by two genes: PS1 and PS2. PS, nicastrin, anterior pharynx-defective (aph-1) and presenilin enhancer 2 (pen-2) form an active part of the γ-secretase complex, while PS form the catalytic core of the complex [18].

Presenilin-1 (PS1) and presenilin-2 (PS2) are considered as the key elements of the γ-secretase complex. The proteins are composed of 9 transmembrane domains containing 467 or 448 amino acids. These domains are autoproteolytically cleaved in the process endoproteolysis to form two ends, each of them having an active aspartate site, which create the catalytic γ-secretase complex site for Aβ. Anterior pharynx-defective (Aph-1) and presenilin enhancer 2 (Pen-2) act as cofactors in the active γ-secretase complex. Aph-1 is a transmembrane protein composed of seven subunits with *N*- and *C*-ends protruding into the lumen and the cytosol. It plays an important role in the initial formation of γ-secretase and carries out the enzymatic function in the final complex. Pen-2 is the smallest membrane protein with two transmembrane domains, in which both the *C*- and *N*-ends point to the lumen. Pen-2 holds an important role in stabilizing PS in the final step of γ-secretase building and also helps in endoproteolysis of presenilins [37].

Nicastrin has been described as the main protein that interacts with presenilins. This part of the γ-secretase complex contains 709 amino acids including glycoprotein with 1 large ektodomain and can serve as the substrate receptor of γ-secretase. Nicastrin is essential for the recognition and processing of the substrate, for the maturation of the γ-secretase complex and its transport to the cell surface [38].

In addition to the amyloidogenic fragment of APP (i.e., sAPPβ), γ-secretase breaks down also a variety of other transmembrane proteins (e.g. Notch). Mutation in PS1 often leads to an increase in the relative production of toxic $A\beta_{1-42}$ peptide, which is hydrophobic and is easily prone to aggregation. This process results in a cascade of pathological events, at the end of which a degenerative damage to neurons comes up. The hypothesis about the influence of PS1 mutations on the creation and subsequent aggregation of $A\beta_{1-42}$ was supported by the results of studies on transgenic mice with an increased production of APP, in which increased formation and accelerated storage of the Aβ deposits occurred. Moreover, the PS mutations always appear in different parts of the protein, so it can be hard to predict what toxic effect due to PS mutation will show up. In this context, however, it is possible that the loss of normal functions of the PS caused by one of the mutations closely correlates with the onset of pathological cascades leading to AD.

The most recent studies have pointed to the loss of function of PS, which is usually associated with the mechanism of AD development. In this respect, it was proved that mice with the knockout genes for both PS proteins exhibit degenerative disruption of the front part of the brain, without the formation and storage of Aβ, although cognitive dysfunctions arise as it is normally observed in AD with the appearance of Aβ in the brain. Similar symptoms can be found in frontotemporal dementia in humans, which is presumably caused by a mutation of the gene for PS1, when amyloidogenesis (i.e., formation of Aβ) does not occur. From the abovementioned information, it follows that neurodegeneration may proceed even without the formation of Aβ [39].

However, PS also plays an important role in many other physiological processes. These processes can be divided into those related with the activity of γ-secretase and those without a close connection with the activity of γ-secretase. It is interesting that some of the inhibitors of γ-secretase increase the production of $A\beta_{1-42}$ in low concentrations while reducing the formation of $A\beta_{1-40}$. A similar effect can be observed as a result of PS mutations [40].

10. Apolipoprotein E and other apolipoproteins

Apolipoprotein (APO) is a general term for denoting proteins which bind with lipids. They play an important role in the regulation of pathological manifestations caused by Aβ. APOE is the main representative of the APO present in the CNS, which is produced and secreted exclusively by astrocytes and microglia. APOE is involved in the transport of lipids between the cells in the CNS, where it physiologically induces the lipid homeostasis, repairs damaged neurons, supports synaptic transmission of excitation and separates specific toxins. The *APOE* gene is encoded by three alleles—*APO-ε2*, *APO-ε3* and *APOE-ε4*. These alleles differ in only two residues at positions 112 and 158. These small differences between the alleles, however, determine their different function. The isoform *APOE-ε2* carries out a neuroprotective function, while the isoform *APOE-ε4*, occurring in a population at about 14%, is associated with a number of diseases. Many studies point to the *APOE-ε4* allele as a risk factor associated with cognitive dysfunction and the onset of AD. The effect of *APOE-ε4* is regulated by cholesterol. The *APOE-ε4* variant has a function of chaperone in relation to the Aβ. The chaperone assists in structural formation of Aβ, but, in fact, it also increases the toxicity of Aβ. The consequences of the relation of *APOE-ε4* to Aβ were demonstrated on transgenic animals, when blocking the interaction of *APOE-ε4* with Aβ significantly reduced the accumulation of Aβ into amyloid deposits. The deposition degree of Aβ depends on the presence of the APOE alleles and descends in a series of *APOE-ε4* > *APOE-ε3* > *APOE-ε2*. Interestingly, the intake of sugary drinks leads to induction of the amyloidogenic process, to distortion of memory functions and increased levels of *APOE-ε4* [41].

11. Aβ and neurodegeneration

A number of studies show that Aβ plays a key role in the onset and progression of AD. But so far, it is still not clear whether the culprit of the onset of dementia is the soluble or insoluble form of Aβ and if the extent of the Aβ impact depends on the localization of this protein in extracellular or intracellular compartments. Current research has revealed a variety of processes in which Aβ plays an important role, for instance, mitochondrial dysfunction, oxidative stress, turmoil and disruption of the transfer function of the membrane. According to the amyloid hypothesis, deposition of Aβ in the brain is the primary cause and controlling force of the degeneration associated with AD, which involves formation of intracellular neurofibrillary tangles and induce the death of neurons [42].

12. Conclusion

AD is a complex neurodegenerative disease which is caused by a number of factors, both biological and environmental. Among these factors, one of the main elements is excessive production of Aβ via amyloidogenic processing of APP, and its subsequent storage in the brain. All of these processes lead to neuronal death, which initializes the outbreak

of dementia and AD with the early onset or sporadic forms with the late start. The genes encoding APP, BACE-1, PS1/2 and *APOE-ε4* thus play a crucial role in the pathogenesis of AD. Besides these genes, it is also worth noting the role of neprilysin and the insulin-degrading enzyme. Both neprilysin and the insulin-degrading enzyme are involved in the elimination of Aβ. The levels of both enzymes are decreased in the brains of patients with AD. Further biochemical, behavioral and clinical studies in this area are, however, necessary in order to develop an effective treatment, whether symptomatic or such that alters the course of the disease or hopefully even heal the disease. BACE-1 is an enzyme that initiates the proteolytic cleavage of APP into smaller fragments of Aβ. According to preclinical and clinical data, BACE-1 is a convenient therapeutic target for the treatment of AD. BACE-1 (−/−) knockout mice are viable but they exhibit a range of neurological symptoms which points to the fact that BACE-1 inhibitors may have serious side effects that are associated with the physiological function of this enzyme. In particular, development of new BACE-1 inhibitors represents a major challenge for the future since only a limited number of these drugs successfully entered clinical trials. From this perspective, the most promising compounds are MK-8931 or E2609 which have been promoted to the II/III phase, while the others are between I and II phases. All the drugs consistently induce a large decrease in spinal cord levels of Aβ, up to 90%. They are usually well tolerated; only testing of two inhibitors of BACE-1 was terminated because of serious side effects. The most discussed question remains to what extent it is beneficial to modulate the activity of BACE-1, and in what phase of the AD it is best to start the treatment [17]. Theoretical knowledge on the mutation of Ala673Thr further shows that 50% of BACE-1 inhibition results in a 20% reduction of the Aβ level. But it still remains unclear to what extent it is necessary to inhibit the activity of BACE-1 if the amyloid plaques are already formed. The amyloid plaques themselves can form many years before the clinical manifestation of the symptoms of dementia. However, in recent years, new theories have emerged posing Aβ on the crossroad [43]. Indeed, in some patients, the presence of Aβ in AD brain does not necessarily mean dementia will break out. Postmortem biopsy showed that older persons can have extensive amyloid burden without any signs of cognitive impairment. Note that it also remains unclear whether these individuals would have developed AD if they had lived longer. Be that as it may, it was proved that the presence of Aβ in cognitively normal persons was prone more rapidly to develop symptoms related to AD [44, 45]. Last but not least, it is not fully understood what the relationship between the quantity of the Aβ deposit and cognitive distortions really is. Nonetheless, everything should be more or less elucidated by the results of ongoing clinical trials, especially those on γ-secretase, which seems to be the most perspective biological target for therapy of AD.

Acknowledgements

This work has been supported by the MH CZ-DRO (University Hospital Hradec Kralove, No. 00179906) and by the grant of the Grant Agency of the Czech Republic No. 16-08554S).

Author details

Jan Korabecny, Katarina Spilovska, Ondrej Soukup, Rafael Dolezal and Kamil Kuca*

*Address all correspondence to: kamil.kuca@uhk.cz

Biomedical Research Centre, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic

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