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Pharmacologists and Alzheimer disease therapy: to boldly go where no scientist has gone before

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Introduction: Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by severe cognitive impairment, inability to perform activities of daily living and mood changes. Acetylcholinesterase inhibitors or NMDA glutamate receptor antagonists are currently used for the treatment of AD, but only the former have weak beneficial effects on cognitive function.
Areas covered: The aim of this review is to provide an overview of the main pharmacological features of both current drugs and new compounds which are still under clinical development for the treatment of AD.
Expert opinion: The discovery of new drugs acting at the early stage of AD could be considered as a 'medical need' and inhibitors of γ-secretase or monoclonal antibodies against Aβ seemed good options. However, inhibitors of γ-secretase, that is, tarenflurbil or semagacestat, were discontinued due to their lack of cognitive improvement or unacceptable side effects. A careful evaluation of the risk:benefit ratio should be considered for monoclonal antibodies since, by increasing the disaggregation of fibrillar amyloid-β-peptide (Aβ), they could increase the neurotoxicity of soluble Aβ oligomers. In conclusion, the discovery of new drugs efficacious in AD subjects is an ambitious goal, however, and one that will require close, active collaboration by pharmacologists, chemists and clinicians.

Keywords: acetylcholinesterase inhibitors, Alzheimer disease, memantine, monoclonal antibodies, β-secretase, γ-secretase

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1. Introduction

Alzheimer disease (AD) is the most common form of dementia among the elderly. Epidemiological data show that the incidence of AD increases with age and doubles every 5 years after 65 years of age with 1275 new cases/100000 persons/year [1]. The prevalence of AD was calculated about 1% in subjects aged 60 – 64 but increases up to 33% in people aged 85 or older, in the Western hemisphere [2]. However, the annual incidence worldwide ranges from 1 to 7% at the ages of 70 and 85, respectively [3]. The Alzheimer Association estimates that it will cost roughly $172 billion annually to care for victims of AD in the near future. Despite intense research into this disease, a cause has yet to be discovered, thus making the search for therapeutic strategies difficult. Furthermore, AD is difficult to initially diagnose, due to sometimes overlapping symptoms of depression and simple forgetfulness as a result of aging. Many treatment regimens for AD are typically not administered until after the patient has begun to show considerable declines in memory and/or cognition, which may be decades after initial changes in brain pathology have begun. It is estimated that AD pathology begins

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Article highlights.

- Alzheimer disease (AD) is the most common form of dementia among the elderly and is characterized by progressive memory loss, inability to perform activities of daily living and mood changes.
- Main drugs used for the treatment of AD are the acetylcholinesterase inhibitors (AChEI) donepezil, galantamine and rivastigmine as well as the *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine.
- AChEI have only a weak effect in terms of improvement of cognitive function and this effect is limited to the first 6 – 12 months of therapy, whereas memantine did not show any significant effect on cognitive performance.
- Other hits/leads/drugs under clinical development are nicotinic receptor agonists, glutamate receptor modulators, γ-secretase inhibitors, monoclonal antibodies, tau inhibitors, serotonin receptor modulators, grow factors and statins.
- Although the promising neuroprotective effects in preclinical models, only a few among the above-mentioned compounds show a significant clinical efficacy in improving cognitive performance in humans.

This box summarizes key points contained in the article.

roughly 20 years before the onset of disease symptoms. Therefore, predicting, diagnosing and treating patients with AD is, collectively, an immense challenge [4].

Phenotypically, AD begins with memory loss. Difficulty remembering recent events or names is a typical symptom of AD, as well as depression. As mentioned previously, diagnosing AD is difficult due to multiple overlapping symptoms with other conditions, and a definite diagnosis of AD can only be made at autopsy. Other symptoms of AD include change in mood, difficulty completing otherwise routine tasks and confusion with time and/or place [1-4].

2. Neuropathology in AD

According to the so-called 'amyloid cascade hypothesis', amyloid-β-peptide (Aβ) plays a main role in the pathogenesis of AD. On the basis of this theory, Aβ is produced by secretase-mediated cleavages of the amyloid precursor protein (APP) [5-7]. The encoding gene for APP is located on the long harm of chromosome 21, the trisomy of which can result in Down syndrome (DS). β-Secretase, an aspartyl protease with a strong homology with the pepsin family, cleaves APP at the extracellular N-terminus, generating an extracellular soluble fragment called sAPPβ and leaving an intramembrane fragment known as C99. Sequentially, γ-secretase, an aspartyl protease formed by four proteins such as nicastrin, presenilin, alphaprotein 1A and presenilin enhancer 2, cleaves the C99 C-terminal end, originating an intracellular fragment (amyloid intracellular domain, AICD) and releasing Aβ (**Figure 1**). The product of these cleavages is either the 40- or 42-amino acid fragment of Aβ [1,8]. A nonamyloidogenic pathway of APP processing also exists, by which, α-secretase cleaves the N-terminus in place of β-secretase at the 17 position, with γ-secretase cleaving the C-terminal end, resulting in a much less toxic product (Aβ 17-42). Other APP processing produces toxic fragments such as C31 and Jcasp [9,10]. Once produced, Aβ forms the core of senile plaques (SP), which are undoubtedly implicated in AD pathogenesis, and the toxicity of this peptide is thought to be heavily dependent on self-association. Aβ monomers are considered to be less toxic, and in one study protective [11] than low molecular weight oligomers of Aβ which has been shown to cause significant adverse cellular events [1,12]. Preclinical evidence demonstrated that Aβ protein oligomers, isolated from the cerebral cortex of AD patients and administered to mice, severely inhibited long-term potentiation, reduced hippocampal dendritic spine density, and disrupted memory of a learned behavior [12]. Insoluble Aβ plaques from AD patients administered to mice were only toxic if plaques were solubilized to release low molecular weight oligomers [12]. In addition, Aβ fibrils were shown to contribute to AD by stimulating the hyperphosphorylation of tau thus increasing the formation of neurofibrillary tangles (NFT) in P301L tau transgenic mice [13]. The link between Aβ and tau was also strengthened by the evidence that two kinases, such as GSK3β and DYRK1A, which are activated by Aβ and APP cleavage products, significantly increased tau phosphorylation [14]. However, since NFT were shown to be characteristic of other diseases, including frontotemporal degeneration and Pick's disease in which Aβ does not have any pathogenetic role, an Aβ-independent hyperphosphorylation of tau was proposed [15]. Recently, the 'amyloid cascade hypothesis' was challenged by another theory which grew up in the attempt to give an answer to several questions which can be summarized as follows: if SP have a so important pathogenetic role in AD, why there was no correlation found between their concentration in brain areas and the degree of dementia, neuronal damage or loss of neurons in humans? Why Aβ deposition in human AD brain was found to be in the same order of magnitude of that detected in normal individuals? Why transgenic mouse models constructed to overexpress Aβ and SP did not exhibit a significant degree of neurodegeneration? Why, in human primary brain cells Aβ42 exhibited a neuroprotective role against type 1 herpes simplex virus? [16,17]. The novel hypothesis developed to reply to these questions is focused on a direct toxic role played by APP and presenilins and support the idea that Aβ is not causative but can be considered as an 'innocent bystander' in AD. Presenilins were shown to be involved in the regulation of many pro-survival intracellular pathways including the PI3K/Akt system, the mytogen-activated protein kinases MEK and ERK, the kinase GSK3β and calcium homeostasis. Additionally, presenilin-1 was demonstrated to promote the degradation of the transcription factor β-catenin and control the release of N-cadherin, the latter playing a pivotal role in hippocampal long-term potentiation [8,18]. In this light, the

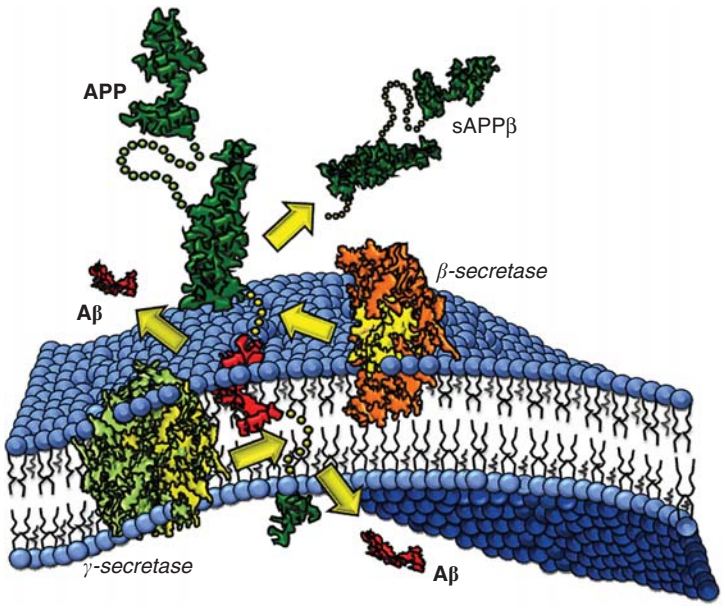

Figure 1. The metabolic pathway leading to the formation of amyloid-β-peptide (Aβ). The amyloid precursor protein (APP) is cleaved by β-secretase, an aspartyl protease, and generates an extracellular soluble fragment (sAPPβ) and a cell-membrane bound fragment called C99. This latter is the substrate for γ-secretase which, in turn, produces Aβ(1-42) fibrils. These fibrils form soluble oligomers which later aggregate in insoluble fibrils, the core of senile plaques.

presenilin-1 mutations found in subjects with familial AD were supposed to interfere with the above-mentioned pro-survival pathways thus leading to neurodegeneration [18]. Independent of the main causative event, AD patients exhibit specific pathological lesions that selectively affect neurons in specific brain regions, in particular the neocortex, entorhinal area, hippocampus, amygdala, basal nucleus of the anterior portion of the thalamus and several brainstem monoaminergic nuclei [19]. These brain areas are endowed with high cholinergic activity and this significantly contributes to loss of cognitive and memory functions characteristic of AD subjects.

2.1 Oxidative stress in AD

Oxidative stress has been implicated in the pathogenesis of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Huntington disease (HD) and Parkinson disease (PD). Hensley *et al.* were among the first to observe the free radical-generating properties of Aβ in brains from AD patients, providing a potential mechanism for pathology-induced neurodegeneration [20]. Later, it was discovered that the free radical toxicity of Aβ may be dependent on free radical transfer reactions via a single Met residue at position 35 of Aβ(1-42). Butterfield *et al.* also showed that mutation of this residue *in vivo* ameliorated global oxidative stress in brains of APP (Swe/Ind) human double mutant transgenic mice [21]. However, others contend that Met35 is not central to the neurotoxicity of Aβ [22].

The oxidative stress hypothesis gained momentum as a possible cause of neuronal death in AD (reviewed in [23]). A wealth of literature exists that multiple regions of the AD brain are overwhelmed by oxidative damage/depletion of antioxidants. The oxidative stress hypothesis in AD is further supported by data showing little/no oxidative damage in neuronal areas that are not affected in AD, such as cerebellum [24]. Our laboratories showed increases in total levels of markers of protein oxidation (protein carbonyls; 3-nitrotyrosine (3-NT)) and lipid peroxidation (protein-bound 4-hydroxy-2-nonenal (HNE)), in brain of subjects with both AD and mild cognitive impairment (MCI) (**Figure 2**) (reviewed in [25]). Furthermore, redox proteomics analysis of brains from MCI and AD patients allowed the identification of proteins with increased carbonylation, as well as HNE and 3-NT modifications [26-32]. Proteins with increased levels of specific oxidation or nitration (oxidative index/total protein) are traditionally observed as having diminished activity; in the case of neurodegenerative disease, oxidized proteins most likely impair neuronal processes that may contribute to cell death in AD/MCI, thus perpetuating the AD phenotype.

In addition to oxidative damage to proteins, lipid oxidation (**Figure 2**) is also a key feature in AD-affected brain regions. Oxidation of membrane-localized lipids diminishes phospholipid levels in neurons, and altering membrane fluidity. Free HNE was detected in brain of AD and MCI patients, and cerebrospinal fluid (CSF) of AD patients [33]. Cultured hippocampal neurons exposed to HNE underwent cell death through impairment of ion motive adenosine triphosphatase activity and alteration of Ca^{2+} homeostasis (reviewed in [23]). Several proteins were identified as having increased bound HNE in

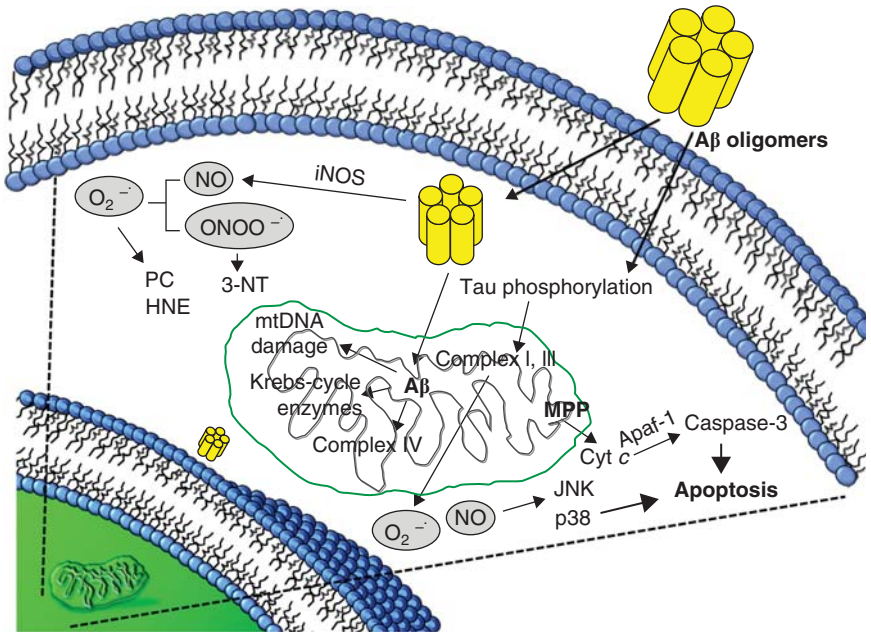

Figure 2. The main intracellular events which contribute to neuronal cell death in Alzheimer disease. Once formed, amyloid-β-peptide (Aβ) aggregates exert toxic effects through: (a) increasing the formation of both reactive oxygen species (ROS), mainly the superoxide anion, and activation of inducible nitric oxide synthase (NOS-2) leading to nitric oxide (NO) generation; (b) mitochondrial impairment by inhibiting important enzymes involved in the respiratory chain and Krebs cycle and causing mitochondrial DNA fragmentation; (c) stimulation of the ionotropic glutamate receptor NMDA and increase of Ca^{2+} overload thus leading to excitotoxic cell death. As a consequence of the first mechanism, NO can react with superoxide generating peroxynitrite ($ONOO^-$). Both ROS and $ONOO^-$ contribute to cell death by oxidizing or nitrating proteins and lipids which generate protein carbonyls (PC), 4-hydroxy-2-nonenal (4-HNE) and 3-nitrotyrosine (3-NT) protein adducts. At the mitochondrial level, Aβ attacks key enzymes involved in the Krebs cycle, such as α-ketoglutarate and pyruvate dehydrogenases, as well as complex IV (cytochrome *c* oxidase), thus impairing glucose metabolism and energy production. In addition, Aβ contributes to the activation of kinases involved in tau hyperphosphorylation. Hyperphosphorylated tau inhibits mitochondrial complex I and synergizes with Aβ to damage mitochondria. As a consequence, the opening of mitochondrial membrane transition pores occurs and causes cytochrome *c* release, caspase-3 activation and apoptotic cell death.

MCI, and AD brains, resulting in decreased enzymatic activity; these proteins are involved in glucose metabolism, mitochondrial electron transport, cytoskeletal maintenance and proteasomal function, all of which are altered in AD relative to brains from healthy controls [25,28,31-32]. In addition to production of HNE, oxidation of arachidonic acid can also yield F2-isoprostanes (F_2-iPs) which were found elevated in AD and patients with probable AD [34,35]. Other products of lipid peroxidation, such as malondialdehyde [36], acrolein [37], thiobarbituric acid reactive substances [38] and F4-neuroprostanes (from docosahexaenoic acid) [39] were also observed to be elevated in various AD reports.

Not surprisingly, products of DNA and RNA oxidation were detected in *postmortem* brain samples from AD patients. An increase in DNA strand breaks in AD brains compared with controls was detected [40]. Significant increases in 8-OH-deoxyguanosine, 8-OH-deoxyadenosine and 5-OH-deoxyuracil in temporal, parietal and frontal lobes as well as elevated 5-OH-deoxycytosine in temporal and parietal lobes were observed in AD [41,42]. Wang *et al.* reported on an increase in oxidized bases in mitochondrial (mtDNA) and nuclear (nDNA), although 10-fold higher levels were observed in mtDNA in late stage AD [43]. In a similar study involving MCI brains, mtDNA and nDNA significant elevations in oxidized base products, indicating that oxidative damage to DNA was an early contributor to disease pathogenesis, were found [44]. Ding *et al.* showed significantly elevated 8-OH-deoxyguanosine in MCI inferior parietal lobule (but not in cerebellum), which correlated with decreased rRNA and tRNA [45], and consistent with altered protein synthesis reported [46].

2.2 Neuronal death

While the involvement of neuronal death undoubtedly contributes to AD progression, the underlying cause of this phenomenon remains elusive. Studies on *postmortem* brains of AD patients indicate the presence of neuronal apoptosis [47]. Sultana *et al.* reported on increased cytosolic

levels of proapoptotic Bcl-2 and caspase-3 in amnestic MCI hippocampus, implicating apoptosis as an early event in AD pathogenesis [48]. Therefore, pathways to programmed cell death may provide therapeutic targets for preventing neurodegeneration. Along with the aforementioned oxidative stress, mitochondrial dysfunction and loss of phospholipids asymmetry [49,50] which are related to oxidative stress, are major pathways leading to the induction of apoptosis.

Mitochondria play a key role in cellular vitality since they are responsible for the generation of adenosine triphosphate (ATP) through oxidative phosphorylation, as well as regulation of intracellular Ca^{2+}. Cytochrome *c* oxidase deficiency in AD has been proposed as a possible cause of increased apoptosis [51]. Another possible mechanism of ATP depletion is oxidation of enzymes involved in glycolysis, the Krebs cycle, or oxidative phosphorylation [26-29,52-55], possibly by Aβ (**Figure 2**). Oxidative inactivation of key enzymes involved in ATP production would undoubtedly impair mitochondrial respiration, cause loss of membrane potentials and ultimately leading to cell death.

Calcium is considered as an important second messenger in neurons, since it regulates membrane excitability, triggers neurotransmitter release at the synapse, mediates gene expression and modulates neuronal growth [56,57]. Therefore, disruption of Ca^{2+} homeostasis within the neuron can have multiple adverse consequences leading to cell death. Ca^{2+} levels in the cytosol are regulated by a cross-talk between voltage-gated Ca^{2+} channels, *N*-methyl-D-aspartate receptors (NMDA), uptake by mitochondria and endoplasmic reticulum stores [56]. Excessive amounts of cytosolic Ca^{2+} are sequestered by mitochondria to a point, at which Ca^{2+} can trigger mitochondrial mechanisms leading to the opening of the mitochondrial permeability transition pore (MPTP) and the release of cytochrome *c* from the mitochondrial matrix to the cytosol, a key event in the intrinsic apoptotic pathway [58]. In AD, Aβ-plasma membrane interactions result in the formation of ion-conducting pores, leading to increased cytosolic Ca^{2+} and elevated vulnerability of neurons to excitotoxicity [59,60]. Furthermore, oligomeric Aβ can cause Ca^{2+}-related toxicity in cultured neurons [61]. Lipid peroxidation, a well-established event in AD and MCI and oxidative modification of NMDA receptors, Ca^{2+} membrane channels, glutamate and glucose transporters would also contribute to elevated cytosolic Ca^{2+} in AD, leading to apoptosis [55,62-63].

3. Available drugs for use in Alzheimer disease

The drugs currently available for the treatment of dementia are acetylcholinesterase inhibitors (AChEI) or NMDA glutamate receptor antagonists [64-69]. The former include donepezil, rivastigmine, galantamine and few others that are still undergoing testing [68]. They are used to increase synaptic levels of acetylcholine, which are reduced as a result of damage to cholinergic neurons in the amygdala, hippocampus and frontal cortex, the brain areas that are responsible for the maintenance of memory. NMDA receptor antagonists, like memantine, are used to prevent/reduce calcium-dependent excitotoxic neuronal cell death [66,67,69]. AChEI produced some degree of improvement in cognitive functions, but their effects were confined largely to patients with mild-to-moderate AD-like dementia, and the most marked effects observed during the first year or so of treatment [70,71]. Thereafter, their efficacy declines progressively and disappears entirely after 2 or 3 years. Attempts were made to increases the efficacy of AChEI by combining them with memantine, but it remains to be seen whether these associations are more effective than the single drugs alone [72,73].

The efficacy of drugs used in the therapy of subjects with mild-to-moderate AD is evaluated using the criteria established by the US Department of Health and Human Services (a component of which is the National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)). As primary efficacy measures the Alzheimer's Disease Assessment Scale-cognitive subscale score (ADAS-cog) or the Mini Mental State Examination (MMSE) are considered standard clinical assessment instruments for determining cognitive function in AD. The ADAS-cog includes 11 individual tests: spoken language ability, comprehension of spoken language, recall of test instructions, word finding difficulty, following commands, naming objects, construction drawing, ideational praxis, orientation, word recall and word recognition [74]. The MMSE includes the following tasks: orientation to time and place, retention of three words, attention and recall of three words, language and visual construction [75]. As a second primary efficacy measure that is often applied is the Clinician's Interview Based Assessment of Change-Plus (CIBIC-plus) patient function is assessed in four general areas (general status, cognitive function, behavior, daily activities) through a subjective interview by a clinician [76].

3.1 Acetylcholinesterase inhibitors

3.1.1 Donepezil

Donepezil, {(±)-2,3-dihydro-5,6-dimethoxy-2-([1-[phenylmethyl]-4-piperidinyl]methyl)-1*H*-inden-1-one hydrochloride}, originated by Eisai Co. Ltd. (Toyko, Japan) and licensed to Pfizer, Teikoku Pharma USA and Teikoku Seiyaku, is extensively used to delay cognitive decline in subject with mild-to-moderate AD. After oral administration, donepezil has an excellent bioavailability and plasma peak concentration is achieved in 3 – 4 h [77]. The drug is tightly bound to plasma proteins and this could account for the prolonged half-life of ~ 70 h [77]. This drug is metabolized by the liver, through the isoforms 3A4 and 2D6 of the cytochrome P-450 (CYP) and is mainly excreted by the kidney even if a small part of the drug is recovered in the feces [77] (the main pharmacokinetic parameters of donepezil are listed in **Table 1**). Important pharmacokinetic interactions occur if donepezil is administered together with inducers or inhibitors of CYP3A4 and CYP2D6, and this could be a common event

Table 1. The main pharmacokinetic parameters of the marketed drugs for AD.

Drugs	Bioavailability *per os* (%)	Plasma protein binding (%)	T_{max} (h)	Half-life (h)	Metabolism	Excretion
AChEI						
Donepezil	Excellent	90	3 – 4	70	Liver (CYP3A4, 2D6)	Kidney (or feces)
Rivastigmine	Good	40	1 14 – 22 (TTS)	1.5 – 2 3 (TTS)	Liver (sulfate conjugation)	Kidney
Galantamine	Excellent	20 – 30	1 – 2	5 – 7	Liver (CYP3A4, 2D6)	Kidney
NMDA receptor antagonist						
Memantine	Excellent	–	6 – 8	60 – 80	–	Kidney

AChEI: acetylcholinesterase inhibitors; AD: Alzheimer disease; NMDA: *N*-methyl-D-aspartate; T_{max}: Time to reach peak plasma concentration; TTS: Transdermal therapeutic system.

considering that very often AD subjects are affected by concomitant diseases (**Table 2**). From a pharmacodynamic point of view, donepezil is a selective, reversible inhibitor of AChE with only a minimum activity against butyrylcholinesterase (BChE) [77]. In addition, donepezil and galantamine (see Section 3.1.3) act as allosteric potentiation ligands on α4- and α7-nicotinic ACh receptors. Through this mechanism, donepezil and galantamine activate pro-survival pathways such as the proto-oncogene Akt and protein Bcl-2 and down-regulate calcium-induced activation of nitric oxide synthase and the further increase of cytotoxic reactive nitrogen species, including peroxynitrite [78].

Since 1996, several clinical studies about the therapeutic role of donepezil were conducted in the USA and Europe, and more than 3000 subjects with mild, moderate or severe AD were enrolled. Early studies were conducted to select the more effective dosage of donepezil in subjects with mild-to-moderate AD [79-82]. These studies were randomized, double-blind and placebo-controlled clinical trials (RCT) and all of them demonstrated the efficacy of donepezil versus placebo to improve cognitive function in AD patients (thus matching primary outcomes). Unfortunately, these studies were not powered enough to detect differences between the 5 and 10 mg/day dose groups and no significant difference in efficacy was found between doses.

In two 12-month clinical studies, the first of which was a placebo-controlled prospective study [83] and the second a RCT [84], 717 patients with mild-to-moderate AD were randomized to receive donepezil at dosage target of 5 mg for 28 days and 10 mg thereafter. The results showed a significant effect of donepezil versus placebo on cognitive performance and activities of daily living as well as an extension of the median time to clinically evident functional decline by 5 months versus placebo [83,84]. A 6-month RCT with a target dose of 5 mg/day donepezil and 10 mg thereafter, suggested the efficacy of this drug to improve cognitive function (CIBIC-plus and MMSE scores) also in subjects with moderate-to-severe AD [85]. *Post hoc* analyses on a population of severe AD patients in nursing home settings, confirmed the efficacy of donepezil on cognitive, functional and behavioral symptoms [86,87]. With regard to the activities of daily living in severe AD patients, Winblad *et al.* [88] described a significant improvement after 6 months of treatment, whereas Black *et al.* [89] reported a lack of efficacy of the drug in two independent RCT. Data obtained by pooling the results of three RCT on the efficacy of donepezil in severe AD patients demonstrated a significant beneficial effect of this agent for cognition, and global function, but no positive effect on behavior [90]. Due to the evidence that statins could have a protective effect on AD (see Section 4.9), donepezil (10 mg/day for > 3 months) was associated with atorvastatin (80 mg/day for 72 weeks), and the overall effect was evaluated in mild-to-moderate AD subjects. The results of the LEADe RCT showed a non-significant effect of donepezil plus atorvastatin on both cognition and global function [91]. Among the adverse effects of donepezil, worthy of mention are chest pain, nausea, emesis and weight loss [79,80,86].

3.1.2 *Rivastigmine*

Rivastigmine [(*S*)-*N*-ethyl-3-[(1-dimethylamino)ethyl]-*N*-methylphenylcarbamate hydrogen], originated by Novartis (Basel, Switzerland) and licensed to Abbott GmbH & Co KG, Biosintetica, Ono Pharmaceutical and Pensa Pharma, is well absorbed by oral route, the plasma protein binding is ~ 40%, the plasma peak concentration is achieved in 1 h and the half-life is ~ 1.5 – 2 h [77]. The metabolism of rivastigmine is rapid and extensive and occurs mainly through cholinesterase-mediated hydrolysis to the NAP-226-90 metabolite which undergoes sulfate conjugation in the liver and is excreted by the kidney [77] (the main pharmacokinetic parameters of rivastigmine are listed in **Tables 1** and **2**). Pharmacodynamically speaking, rivastigmine is not a selective inhibitor of AChE because it also inhibits BChE with equal potency [77]. In addition, rivastigmine forms a carbamoylated complex with both AChE and BChE, characterized by a covalent bond, which makes the complex more resistant to the hydrolysis and this likely affects the half-life of this drug [77]. The main adverse effects include dizziness, anorexia, nausea, vomiting and dyspepsia [92].

Significant differences in global function and measures of cognition favored rivastigmine in subjects with mild-to-severe

Table 2. Pharmacological interactions involving currently available drugs for AD.

Drugs	Drugs which enhance the metabolism	Drugs which inhibit the metabolism
Donepezil, Rivastigmine	*CYP3A4 inducers*: barbiturates, glucocorticoids, macrolide antibiotics, phenytoin, rifampin *CYP2D6 inducers*: St. John's wort, rifampin	*CYP3A4 inhibitors*: diltiazem, erythromycin, fluconazole, grapefruit juice (furanocumarins), ketoconazole, ritonavir, troleandomycin *CYP2D6 inhibitors*: quinidine, paroxetine
Galantamine	*SULT inducers:* carbamazepine, genistein, green tea, methotrexate	*SULT inhibitors*: curcumin, diflunisal, mefenamic acid, nimesulide, salicylates
Memantine	*UGT inducers:* carbamazepine, phenobarbital, rifampin	*UGT inhibitors:* amitriptyline, curcumin, diclofenac, fluconazole, mefenamic acid, oxazepam, valproic acid

AD: Alzheimer disease; CYP: Cytochrome P-450; SULT: Sulfotransferase(s); UGT: Uridinediphosphoglucuronosyl transferase(s).

AD. In a 26-week RCT, 725 subjects with mild-to-moderately severe AD received two dose regimens of rivastigmine: 1 – 4 mg/day or 6 – 12 mg/day. Only subjects treated with rivastigmine 6 – 12 mg/day maintained their baseline levels of cognitive performance and demonstrated favorable and significant differences in cognition, participation in activities of daily living and global evaluation [93]. In the follow-up study, a larger difference was seen in ADAS-cog scores between the rivastigmine (6 – 12 mg/day) group versus the placebo group at 52 weeks but only subjects originally treated with rivastigmine 6 – 12 mg/day had better cognitive function [94]. A retrospective analysis suggested efficacy of rivastigmine in subjects with moderate-to-severe AD. Data pooled from three 6-month RCT demonstrated that moderate-to-severe AD patients treated with rivastigmine 6 – 12 mg/day had better cognitive performance compared with the control group after 6 months from treatment [95]. These results suggested that rivastigmine provides clinical benefit to patients with moderate-to-severe AD. Currently, an alternative route of administration was proposed for this drug, and the rivastigmine patch is the first transdermal treatment to be approved for mild-to-moderate AD in the USA and Europe. The main reason that the transdermal route was approved is based on the better pharmacokinetic profile of rivastigmine which, from the dermal patch, is continuously delivered into the bloodstream, thus avoiding the fluctuations in plasma concentration due to the oral route of administration [96,97]. The first studies designed to test the efficacy of transdermal rivastigmine were 6-month RCT which demonstrated that a 10 cm^2 patch, which corresponds to 9.5 mg/day rivastigmine, provided similar efficacy to 12 mg/day rivastigmine capsule, and also guaranteed a threefold reduction in reports of nausea and vomiting [98,99]. An updated paper compared the results from three RCT with rivastigmine patches versus capsules and reported that the former have better safety and tolerability profiles than the latter, and the risk of skin reaction can be decreased simply by rotating patch location [100]. With regard to cognitive performance, a recent paper demonstrated that a 10 cm^2 rivastigmine patch improved cognitive and functional performance in AD patients [101]. The rivastigmine patch is well tolerated and only mild adverse effects (erythema and pruritus) were recorded [96,102].

3.1.3 *Galantamine*

Galantamine (4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol), originated by Sanochemia Pharmazeutika (Vienna, Austria) and licensed to Janssen Pharmaceutical KK, is a tertiary alkaloid that has been isolated from various plants, including narcissus species and the Caucasian snowdrop (*Galanthus nivalis*) and this drug is approved for clinical use in AD. Similarly to donepezil, galantamine has an excellent bioavailability after oral administration, but lower protein binding (15 – 30%) and both shorter time-to-reach peak concentration (1 – 2 h) and half-life (5 – 7 h) [77]. Galantamine is metabolized by the liver, through the isoforms 3A4 and 2D6 of the CYP and is mainly excreted by the kidney (**Table 1**) [77]. Like donepezil, important pharmacokinetic interactions could occur if galantamine is administered together with inducers or inhibitors of CYP3A4 and CYP2D6 (**Table 2**) [77]. Galantamine shares with donepezil also the ability to stimulate Akt and Bcl-2 pathways and inhibit NO-induced cytotoxicity, and thus counteract neuronal death [78].

Tariot *et al.* investigated the efficacy and tolerability of galantamine (8, 16 or 24 mg/day) in a 5-month RCT involving subjects with mild-to-moderate AD [103]. Only subjects treated with the two higher doses of galantamine had significant benefits in the cognitive, functional and behavioral symptoms of AD as compared with placebo [103]. These results were further confirmed in two RCT carried out by other investigators [104,105]. However, *post hoc* analysis of a 5-month RCT suggested that 16 mg/day is the optimal dosage for galantamine in patients with mild AD, whereas, patients with moderate AD appear to gain additional benefit from galantamine 24 mg/day [106]. In subjects with severe AD, 24 mg/day galantamine for 6 month exhibited a significant improvement in cognitive function, but did not demonstrate any benefit in overall activities of daily living [107].

3.2 NMDA glutamate receptor antagonist

3.2.1 *Memantine*

Memantine (3,5-dimethyladamantan-1-amine), originated by Children's Medical Center Corp. (Boston, MA, USA) and

licensed to Merz Pharma, binds NMDA receptor channels, thereby inducing a non-competitive block. After administration of an oral dose, memantine is almost completely absorbed, reaches the peak plasma values in 6 – 8 h and its half-life is 60 – 80 hours (**Table 1**) [108]. About 50% of the drug is excreted unchanged by the kidney, whereas the remainder is converted into glucuronide derivatives and excreted in the urine (**Table 1** and **2**) [108]. The main side effects of memantine are dizziness, constipation, cataracts, nausea, dyspnea, confusion, headache and urinary incontinence [109]. Caution should be used in the case of concomitant administration of memantine and inducers or inhibitors of UGT-glucuronosyltransferase(s) (**Table 2**).

Three large multicenter 6-month RCT confirmed the efficacy of oral memantine (20 mg/day) alone or in combination with donepezil, in moderate-to-severe AD [110-112]. The results from these RCT demonstrated that memantine improved only the activities of daily living without any significant effect on cognitive function [110,112]. When administered in moderate-to-severe AD patients already treated with donepezil, memantine improved cognitive function and the activities of daily living [111]. In a recent study, subjects with mild-to-moderate AD randomized to receive either donepezil or memantine for 6 months, did not show any change in cognitive function as well as in neuronal density (evaluated by measuring *N*-acetyl aspartate, myo-inositol and choline) in temporal, prefrontal, posterior cingulated and occipital areas of the brain [113]. Recently, Schneider *et al.* analyzed the results of three RCT and demonstrated the lack of efficacy of memantine to improve cognitive function and activities of daily living in mild AD patients [114]. Memantine was also associated with both oral and transdermal rivastigmine in mild-to-moderate AD subjects. In a 25-week open-label study, subjects with mild-to-moderate AD were treated with memantine alone or in the presence of rivastigmine patches (4.6 mg/day rivastigmine patches for 4 weeks and then with 9.5 mg/day patches for further 20 weeks) [115]. The results showed that changes in cognitive and global function were similar between the two arms of treatment, whereas the activities of daily living scores worsened in both the groups, even more than in those patients treated with memantine alone [115]. The incidence of adverse effects did not significantly increased when memantine was given concomitantly with oral or transdermal rivastigmine and the most common adverse effects were nausea, vomiting and dizziness [115,116].

4. Drugs still under development

4.1 Acetylcholinesterase inhibitors

4.1.1 *Latrepirdine*

Latrepirdine [2,3,4,5-tetrahydro-2,8-dimethyl-5-(2-(6-methyl-3-pyridyl)ethyl)-1H-pyrido(4,3-b)indole] also known as dimebon or dimebolin, was originated by Medivation (San Francisco, CA, USA) and licensed to Pfizer. Dimebon was initially developed as a orally active non-selective antihistamine drug [117,118], but due to the development of newer and safer antihistamine drug, it was withdrawn from the market. The interest on latrepirdine was renewed at the beginning of the 2000s when this agent exhibited neuroprotective effects in preclinical models of AD and Parkinson disease [119-121]. This evidence prompted neurologists to design *ad hoc* clinical trials to evaluate the effect of dimebon in subjects with mild-to-moderate AD. In a RCT, Doody *et al.* demonstrated that dimebon 20 mg three times a day significantly improved cognitive function (measured as ADAS-cog score) over 26 weeks of treatment [122]. In the extension phase of the trial, patients taking dimebon were followed-up for further 26 weeks and they still exhibited a significant improvement in cognitive function (ADAS-cog, MMSE and CIBIC-plus scores) with respect to those treated with placebo [122]. More recently, a larger RCT (CONNECTION) involved 598 subjects with mild-to-moderate AD treated with dimebon 5 or 20 mg three times a day. This study demonstrated that 6-month treatment with both the doses of dimebon did not improve cognitive and global functions in AD subjects, and the development of dimebon was partially discontinued [123]. However, the Phase III CONCERT study which evaluated the effect of dimebon in AD patients treated concomitantly with donepezil is still ongoing (**Table 3**).

4.2 Nicotinic receptor agonists

4.2.1 *Ispronicline*

Ispronicline, originated by R. J. Reynolds Tobacco Co. (Winston-Salem, NC, USA) and licensed to AstraZeneca, is an oral active $\alpha4\beta2$ nicotinic acetylcholine receptor-selective agonist with neuroprotective effects in humans [124]. After oral administration, ispronicline reaches the peak plasma concentration after 1 – 2 h and the terminal half-life is ~ 3 – 5 h (single doses) and ~ 3 – 9 h (repeated doses) [125]. In July 2009, AstraZeneca announced that the development of a novel $\alpha4\beta2$ receptor agonist, named AZD 1446, has been prioritized over further development of ispronicline in AD.

4.2.2 *RG 3487*

RG 3487 (originated by Memory Pharmaceuticals which was acquired by Roche (Basel, Switzerland) in 2009) is a partial agonist of the α-7 nicotinic acetylcholine receptor and 5-HT3 antagonist [126]. Preclinical data showed that this agent improved the attention and increased the accuracy performance in the rat [126].

In a Phase I RCT, 15 mg/day RG 3487 for 13 days improved the quality of episodic secondary memory in healthy volunteers [127]. Data from Phase II RCT indicated that RG 3487 (5, 15 and 50 mg, once daily) ameliorated cognitive function in mild-to-moderate AD subjects [128]. On February 2011, RG 3487 development was discontinued in the European Union, USA, Argentina, Australia and Canada.

4.2.3 *EVP-6124*

EVP-6124, an orally active selective α-7 nicotinic receptor agonist, developed by Bayer HealthCare (Leverkusen, Germany)

Table 3. AD drug development*.

Compound	Phase of development		
	USA/Europe	Asia	Japan
Donepezil	Marketed	Marketed	Marketed
Rivastigmine	Marketed	Marketed	Marketed
Galantamine	Marketed	Marketed	Marketed
Memantine	Marketed	Marketed	Marketed
Dimebon	Discontinued‡ III§		
Ispronicline	Discontinued	Discontinued	Discontinued
RG 3487	Discontinued		
EVP-6124	II		I
EVT 101	Discontinued	Discontinued	Discontinued
LY451395	II		
Semagacestat	Discontinued	Discontinued	Discontinued
Tarenflurbil	Discontinued		
Bapineuzumab	II – III	III	III
Solanezumab	II – III	III	III
MABT 5102A	I		
Gantenerumab	II	II	I
Methyltioninium	II		
Davunetide	II		
PRX 03140	II		
Lecozotan	Suspended	Suspended	Suspended
CERE 110	II		
Cerebrolysin	Marketed	Marketed	

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‡Late-stage AD.

§Early-stage AD.

AD: Alzheimer disease.

and licensed to EnVivo Pharmaceuticals, improved cognitive tasks in a preclinical rat model of dementia. EVP-6124 has a good plasma:brain ratio and the half-life is suitable for a once-daily dosing [129]. In a Phase Ib/IIa RCT, EVP-6124 (0.1 – 0.3 – 1 mg/day for 1 month) was safe and well tolerated in mild-to-moderate AD patients stabilized on AChEI (donepezil or rivastigmine) [129]. In addition, this drug exhibited pro-cognitive effects in various cognitive domains, such as non-verbal learning, memory and executive function [129]. A second Phase IIb RCT was initiated in May 2010 by EnVivo Pharmaceuticals in subjects with mild-to-moderate AD to specifically evaluate cognitive function through specific tests such as the ADAS-cog scale [130].

4.3 Glutamate receptor modulators

4.3.1 EVT 101

EVT 101, originated by Roche (Basel, Switzerland) and licensed to Evotec AG, is an antagonist at the NR2B subunit of the NMDA receptor. The initial indication of this agent was treatment-resistant depression, but it was also active in subjects with AD [131]. After oral administration, EVT 101 is well absorbed and the half-life is about 11 h. This drug efficiently penetrates the blood–brain–barrier (BBB) and reaches CSF concentrations at levels predicted to inhibit NR2B receptor to a greater extent than those concentrations required for memantine [132,133].

In a Phase Ib study, EVT 101 showed potential beneficial effects on brain function in 19 healthy volunteers. The administration of this drug improved cerebral blood flow in specific region of the cortex, but this effect was not paralleled by a significant amelioration of cognitive functions [132]. The drug is well tolerated and no severe adverse effects compared with the placebo group were reported.

4.3.2 LY451395

LY451395 (Eli-Lilly, Indianapolis, IN, USA) is an AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptor potentiator. This drug is administered by oral route, reaches the peak plasma concentration after 1.5 – 3 h and the half-life is about 9 – 11 h. At the therapeutic dosage of 1 – 5 mg, LY451395 penetrates the BBB with a plasma:CSF ratio of ~ 44:1 [134].

In subjects affected by mild-to-moderate AD, 0.2 – 1 mg b.i.d. LY451395 for 28 days did not have any effect on cognitive function up to 8 weeks from treatment [135]. In March 2009, Eli-Lilly initiated a Phase II RCT of LY451395 as a

treatment for aggression and agitation in AD subjects that was completed in November 2010.

4.4 γ-Secretase inhibitors

4.4.1 *Semagacestat*

Semagacestat (LY450139, Eli-Lilly, Indianapolis, IN, USA) belongs to the family of γ-secretase inhibitors, and this agent was shown to reduce the rate of formation of Aβ *in vitro* and *in vivo*. After oral administration, semagacestat achieves peak plasma concentration after ~ 0.5 h and the half-life is ~ 2.4 h [136]. This agent is metabolized by the liver yielding two main derivatives named M2 (the amine metabolite) and M3 (the benzylic hydroxy metabolite), which are excreted through the urines and feces [136].

A Phase II RCT showed that subjects affected by mild-to-moderate AD and treated *per os* with semagacestat 30 mg for 1 week followed by 40 mg for further 4 weeks exhibited a significant reduction in plasma Aβ (38.2%) levels, whereas in the CSF there was not any significant change [137]. In the same study, semagacestat did not improve cognitive function [137]. A greater reduction in plasma Aβ (58.2 – 64.6%) was obtained in a dose-escalation RCT with semagacestat 60 mg/day for 2 weeks followed by 100 mg/day for 6 weeks and 140 mg for additional 6 weeks [138]; even in this study, semagacestat failed to decrease Aβ CSF concentration or improve cognitive function [138]. In August 2010, Eli-Lilly decided to discontinue the clinical development of semagacestat. Results from two Phase III trials demonstrated that mild-to-moderate AD patients treated with semagacestat (60 – 140 mg/day *per os* for 21 months) did not exhibit any beneficial effect on cognitive function with respect to the placebo group; rather, patients in the active group had an increased risk to develop skin cancer [139].

4.4.2 *Tarenflurbil*

Tarenflurbil, originated by Loma Linda University Medical Center (Loma Linda, CA, USA) and licensed to Encore Pharmaceuticals, PAZ GmbH, is the *R*-enantiomer of the non-steroidal antinflammatory drug flurbiprofen. Tarenflurbil (400 – 1600 mg for 21 days) was shown to inhibit γ-secretase, and decrease cerebral levels of Aβ in healthy elderly individuals [140]. Pharmacokinetic analysis revealed that tarenflurbil given *per os* reaches peak plasma concentration in 1 – 3 h and the half-life is 2 – 8 h.

In a Phase II RCT, 800 mg tarenflurbil twice per day for 24 months improved the activities of daily living and global function but did not improve cognitive function in subjects with mild AD [141]. No effect on measures of daily activities, global function and cognitive performance was shown in patients with moderate AD [141]. These results were confirmed by a recent Phase III RCT, which revealed no significant improvement in the cognitive functions of patients with mild AD after 18 months of treatment with tarenflurbil at a dose of 800 mg b.i.d. [142]. The reason why tarenflurbil did not improve cognitive performance even if it reduced Aβ cerebral levels is still unknown. This finding corroborates the hypothesis mentioned earlier in this paper and relates to the reduced pathogenetic role played by Aβ in AD. On the basis of these results, the development of tarenflurbil was discontinued.

4.5 Monoclonal antibodies

4.5.1 *Bapineuzumab*

Bapineuzumab, originated by Elan Corp. (Dublin, Ireland), is a humanized monoclonal antibody administered by intravenous infusion and was designed to target the N-terminus of Aβ in the brain. Bapineuzumab strongly binds to fibrillar Aβ and is hypothesized to remove Aβ from the brain and prevent or reverse progression of AD [143-145]. Few data about bapineuzumab pharmacokinetics in humans are available. After intravenous administration, bapineuzumab volume of distribution was 49 – 80 ml/kg, total body clearance was 0.07 – 0.09 ml/h/kg and half-life ranged from 21 to 33 days [146,147]. The maximal plasma value for Aβ reached about 24 h following bapineuzumab infusion [146].

In a recent Phase II RCT, the safety/tolerability and efficacy profile of bapineuzumab (0.15, 0.5, 1.0 and 2.0 mg/kg) were tested in 234 subjects with mild-to-moderate AD [148]. In this study, the administration of bapineuzumab failed to meet the primary efficacy endpoint, namely the improvement of cognitive function [148]. However, *post hoc* analyses showed statistically significant clinical benefits associated with bapineuzumab on both cognitive and functional endpoints only in non-carriers of the Apolipoprotein E4 (ApoE4) subgroup [148], but the clinical importance of such a result needs further evaluation. Vasogenic edema, more frequent in ApoE4 carriers, was the main side effect in the bapineuzumab group [145,148]. Other adverse effects were headache, nasopharyngitis, fatigue, diarrhea, urinary tract infection, falls, abrasions and muscle spasm [148].

Phase I and Phase II trials about a new formulation of bapineuzumab for subcutaneous administration began in 2007 and 2008, respectively, and the results are expected in the coming months.

4.5.2 *Solanezumab*

Solanezumab (Eli-Lilly, Indianapolis, IN, USA) is the humanized analog of the murine antibody m266.2. Solanezumab differs from bapineuzumab mainly in the pharmacodynamics. Due to its ability to recognize a distinct epitope in the middle portion of the peptide, solanezumab binds not only to full-length Aβ but also several truncated forms of the peptide [144,149]. In addition, solanezumab selectively binds to soluble Aβ with very low affinity for the fibrillar form [143,144]. Similarly to bapineuzamab, solanezumab has a prolonged half-life ranging from 24 to 50 days [150].

The only randomised, double-blind, placebo-controlled study published showed that a single dose of solanezumab (0.5 – 10 mg/kg) significantly increased Aβ total (bound plus unbound) levels in both plasma and CSF of subjects with mild-to-moderate AD [150]. However, despite the increased

clearance of Aβ from the brain, solanezumab did not improve cognitive functions in these patients [150]. Importantly, solanezumab administration did not result in meningoencephalitis [150] but originated vasogenic edema [151].

4.5.3 Other monoclonal antibodies

Other monoclonal antibodies under development are: MABT 5102A identified from a collaboration between AC Immune Ltd (Lausanne, Switzerland) and Genentech (Roche, Basel, Switzerland), and Gantenerumab identified from MorphoSys (Martinsried/Planegg, Germany) in collaboration with Roche [152,153]. Both these agents are currently in Phase I or II on safety and efficacy are still ongoing.

4.6 Tau inhibitors

4.6.1 Methyltioninium and davunetide

Methyltioninium, an alternate name for methylene blue, prevents tau aggregation processes by blocking the aggregation of tau oligomers and their conversion into paired helical filaments [154]. In addition, methylthioninium dissolves tau aggregates into short truncated monomers that are further cleared efficiently through the proteasomal system [155].

Davunetide is a peptide composed of eight aminoacids (Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln) [156,157]. This agent is derived from an endogenous brain protective protein (activity-dependent neuroprotective protein (ADNP)) and it is able to cross the BBB, thus accumulating in the CNS [157]. Several preclinical studies demonstrated a neuroprotective role for davunetide due to a significant reduction in both hyperphosphorylated and insoluble tau [158,159]. In addition, davunetide reduced Aβ 1-40 and Aβ 1-42 peptide levels in the brain of a transgenic mouse model of AD [160,161]. An interesting aspect that encouraged the development of this drug is its ability to cross the BBB even after endonasal administration [157,160] with an half-life of 2 h in the rat. The good bioavailability of intranasal davunetide was confirmed in humans and is consistent with daily or twice-daily dosing [162].

Several clinical studies about the potential therapeutic use and safety profile of both methyltioninium and davunetide in AD are still ongoing, and the results have not been released yet. Promising is the possibility to administer davunetide by intranasal route rather than intravenously, thus reducing the discomfort and increasing the compliance of AD patients.

4.7 Serotonin receptor modulators

4.7.1 PRX 03140

PRX 03140, identified by EPIX Pharmaceuticals (Lexington, MA, USA) and further acquired by Nanotherapeutics, is a small molecule with partial agonist activity for the type 4 serotonin (5-HT_4) receptor. In preclinical studies, PRX 03140, by stimulating the 5-HT_4 receptor, increased the efflux of ACh and elevated brain-derived neurotrophic factor in the basal forebrain of aged rats [163,164]. Through these multiple mechanisms, this agent improved working memory in aged rats and enhanced visuospatial memory performance of aged beagle dogs [165].

In a Phase I RCT, PRX 03140 showed a good tolerability in both healthy volunteers and elderly patients aged 65 – 80 years [166]. In a later Phase IIa RCT, 80 patients with mild AD were treated with PRX 03140 (50 – 150 mg/day by oral route for 14 days) or in combination with donepezil, and the cognitive performance assessed [167]. The results of this study showed that 150 mg/day PRX 03140 increased cognitive function and induced modifications in brain wave activity (alpha:theta ratio) similar to those observed with AChE inhibitors [167].

4.7.2 Lecozotan

Lecozotan, initially discovered by Wyeth (currently Pfizer, New York, NY, USA), is a potent and silent antagonist of the 5-HT_{1A} receptor [168]. This drug potentiated the stimulated release of glutamate and ACh in the rat dentate gyrus and CA1 hippocampal regions, respectively [169]. Due to the beneficial effects on biochemical pathways involved in cognitive performance, lecozotan is currently being evaluated in mild-to-moderate AD patients. After oral administration, lecozotan is rapidly absorbed, almost completely bound to plasma proteins, achieves peak plasma concentrations in < 1 h and the elimination half-life ranges from 6 to 9 h and 9 to 11 h in healthy young and old subjects, respectively [170]. The degree of receptor occupancy is dose-dependent in the range 0.5 – 5 mg, and it is higher in old or AD subjects with respect to young ones [171]. Despite these promising pharmacological properties, lecozotan did not improve cognitive function [170]; among the other clinical correlates, 10 mg lecozotan significantly increased the adrenocorticotropin hormone and prolactin plasma levels as early as 1 h and 2 h from the administration [170].

4.8 Growth factors

4.8.1 CERE 110

CERE 110, developed by Ceregene (San Diego, CA, USA), is a gene therapy and employs an adeno-associated viral vector system to deliver the gene for the nerve growth factor (NGF) to selected brain regions [172,173]. In fact, NGF was shown to prevent the death of cholinergic neurons and reverse memory loss [174-176]. Preclinical studies in primates demonstrated that NGF gene can remain active for at least 1 year [176]. This gene therapy is not considered as a cure for AD, but it conceivably could protect or restore damaged brain cells and alleviate memory loss.

Interim results from a pilot open trial in subjects with mild-to-moderate AD suggested that a single administration of CERE 110 in the nucleus basalis of Meynert, *via* stereotactic surgery, is well tolerated, even if two patients experienced hemorrhage during the surgical procedure which was subsequently modified to eliminate this problem [177]. In a Phase I, open-label trial in six patients with mild-to-moderate AD, CERE 110 gene therapy was associated with a decrease in cognitive decline and an increase in brain metabolism [177]. However, due to the low sample size of this open-label study, these results need to be carefully considered.

4.8.2 *Cerebrolysin*

Also known as FPF 1070 (developed by Ebewe Neuro Pharma (currently Sandoz International GmbH, Holzkirchen, Germany) and marketed by Abbott GmbH & Co.), cerebrolysin is a peptidergic drug which accelerates neural growth and survival of cholinergic neurons [178].

In subjects with mild-to-moderate AD, cerebrolysin (10 – 30 – 60 ml) given intravenously 5 days/week for the first 4 weeks and then two infusions per week for 8 weeks, significantly improved cognitive performance and global function [179]. This effect was achieved only with the 10 ml dose, whereas the 30 and 60 ml doses improved the global outcome but failed to ameliorate cognition [179]. Other studies reported on the ability of cerebrolysin (30 ml i.v. 5 days/week for 4 – 6 weeks) to improve the activities of daily living in AD patients [180,181]. In subjects with mild-to-moderately severe AD, cerebrolysin (30 ml i.v. once daily 5 days/week for 4 weeks) ameliorated cognitive function, non-cognitive psychiatric symptoms and the activities of daily living [182]. In 2007, Wei *et al.* performed a meta-analysis and concluded that cerebrolysin markedly improved clinical global impression in patients with mild-to-moderate AD, whereas no convincing evidence supports its benefit in cognitive function [183].

4.9 Statins

Statins are common drugs used in dyslipidemias. They reversibly inhibit the hydroxyl-methyl-glutaryl-CoA (HMG-CoA) reductase thus inhibiting the transformation of HMG-CoA into mevalonate, the first step in the cholesterol biosynthesis. Recent preclinical evidence supported the possible use of atorvastatin in AD and the rationale was related to the ability of this drug to counteract oxidative stress in specific brain areas, such as parietal cortex, without a significant interaction with the cholesterol biosynthetic pathway [184,185]. That said, clinical evidence did not confirm such results, and atorvastatin (80 mg for 72 weeks) failed to ameliorate both cognition and global function in mild-to-moderate AD subjects [91,186].

5. Conclusions

Although the enormous *in vitro* and *in vivo* lines of evidence produced over the last 25 years, several issues in the pathogenesis of AD remain still poorly understood. As mentioned earlier in this article, two pathogenetic hypotheses are currently debated, each of which has strong pros and cons. One of the reasons which contributed to these diverging theories is the many experimental models used by the investigators to study the cellular events which characterize AD. Human or rodent cell lines, transgenic mice overexpressing the genes for APP or PS1, *ex vivo* studies performed on human tissues are only a small example of the experimental systems used to produce evidence in favor of or against each theory. In this light, many efforts are still needed in order to develop better animal models that can more reliably predict efficacy. However, it is also important to underlie that preclinical evidence cannot be translated to humans and drugs which showed a terrific beneficial effects when tested in laboratory animals did not provide the same effects in humans. In addition to these 'general' concerns, other 'specific' criticisms have to be considered. Due to the peculiar anatomical localization of brain, protected by the BBB, and the clinical history of AD, which is often discovered at a late stage of the disease, both current available drugs and novel lead compounds still under development, have only a slight impact on the progression of the disease. The discovery of new 'pathogenetic' drugs acting at the very beginning stage of the disease, preventing and/or delaying the oxidative/nitrosative stress modifications of brain tissue could be considered as a 'medical need'. It is an ambitious goal, however, and one that will require close, active collaboration by pharmacologists, chemists and clinicians.

6. Expert opinion

Although many efforts done by independent scientists and pharmaceutical companies to discover new drugs effective in AD, only AChEI and NMDA antagonists are currently available in the market. From a pharmacological point of view, AChEI should be considered as 'symptomatic drugs' since they improve cognitive function in patients suffering from mild-to-moderate AD, whereas memantine has to be considered a 'pathogenetic drug' due to its ability to reduce exitotoxic cell death in neurons. However, clinically speaking, AChEI do not cause a long-lasting improvement in memory and cognitive function as they have a narrow 'therapeutic window' restricted to the first 6 – 12 months of therapy. With regard to memantine, its clinical benefit either as monotherapy or administered together with donepezil, is still questioned. That said, it is straightforward to understand why scientists are trying to fight against AD by developing new drugs which act early during the development of the disease in order to prevent or reduce the brain damage and the following memory and cognitive loss. In this light, drugs acting on Aβ production and/or clearance seemed good options. In spite of strong evidence that Aβ(1-42) can replicate oxidative modification and dysfunction of key transporters, synaptic elements and mitochondria as observed in AD brain (see [25,187] for reviews), as well as the observation that familial AD is associated with elevated Aβ(1-42) in brain, concern that Aβ-centric therapeutic strategies may not be appropriate for AD exists. This concern is also based on the lack of neuronal loss in mouse models of AD in which human mutated Aβ is overexpressed. Indeed, as outlined below or elsewhere [188,189], drugs that have proven useful in AD mouse models have failed in clinical trials in patients. However, there has been a disconnect between prevention and treatment modalities in drug treatment. Namely, the administration of drugs at earlier times prior to pathology has not been permitted in human trials, and in the case of AD, extensive pathology, including neuronal loss, has already occurred.

The occurrence of skin cancer secondary to semagacestat treatment, highlights the potential side effects due to unwanted interactions of the drug with unspecific pathways. Semagacestat inhibited not only γ-secretase but also other substrates, as Notch, and this was responsible for skin cancer after prolonged sun exposure. This kind of problem, which blocked the clinical development of a new drug, could be avoided by a careful screening of the potential interactions of the drug with known substrates responsible for toxic effects during the preclinical phase. However, it is not reasonable to hypothesize the study of all the interactions between the new drug and toxicity pathways, therefore this potential hazard is not completely avoidable. In the case of monoclonal antibodies against Aβ, a careful balance of the risk:benefit ratio should be considered. Indeed, bapineuzumab and solanezumab, by binding fibrillar Aβ and facilitating its disgregation into oligomers, could increase the neurotoxicity of low molecular weight Aβ thus leading to brain damage. In addition, bapineuzumab-treated patients worsened on their MMSE test [145] and this suggests that Aβ clearance have a negative impact on cognitive function.

Finally, the ultimate problem is that a definitive cause of AD is not known. However, the updated amyloid cascade hypothesis is a unifying paradigm to investigate. Patients of this devastating disease and their families deserve our best efforts, and until a better strategy appears, Aβ and its sequelae are appropriate to target pharmacologically. In the meantime, greater efforts to find the one certain cause of AD should be accelerated.

Declaration of interest

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