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Review article

Progress in drug development for Alzheimer's disease: An overview in relation to mitochondrial energy metabolism

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ABSTRACT

Current possibilities of Alzheimer's disease (AD) treatment are very limited and are based on administration of cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and/or N-methyl-D-aspartate receptor antagonist, memantine. Newly synthesized drugs affect multiple AD pathophysiological pathways and can act as inhibitors of cholinesterases (AChE, BuChE), inhibitors of monoamine oxidases (MAO-A, MAO-B), modulators of mitochondrial permeability transition pores, modulators of amyloid-beta binding alcohol dehydrogenase and antioxidants. Effects of clinically used as well as newly developed AD drugs were studied in relation to energy metabolism and mitochondrial functions, including oxidative phosphorylation, activities of enzymes of citric acid cycle or electron transfer system, mitochondrial membrane potential, calcium homeostasis, production of reactive oxygen species and MAO activity.

Contents

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by impairment of cognitive functions and memory loss. AD is the most common cause of dementia. Age is the main risk factor for development of the disease. AD involves two major neuropathological hallmarks: presence of extracellular amyloid β-peptide (Aβ) deposits (senile plaques), and aggregates of hyperphosphorylated tau protein (neurofibrillary tangles). Aβ and tau protein abnormalities cause neuronal dysfunctions and cell death. Although Aβ accumulation [1] along with the tau hyperphosphorylation is the most proposed pathogenetic mechanism

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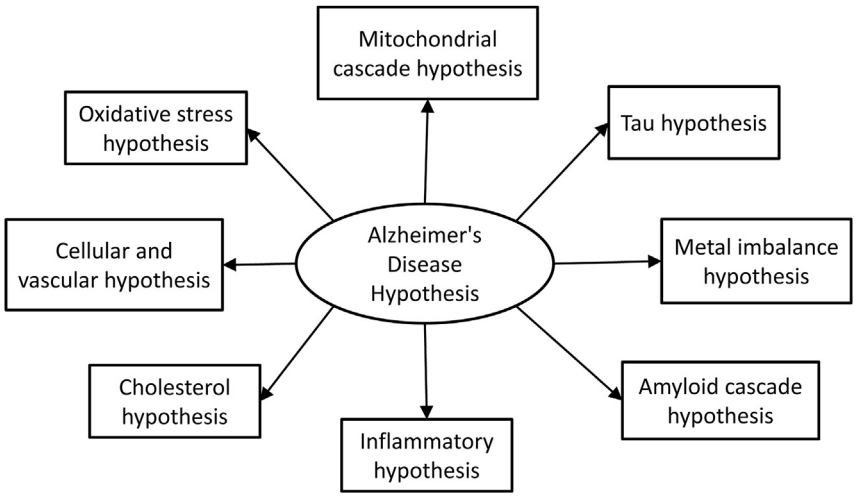

Fig. 1. Alzheimer's disease hypotheses.

[2], mitochondrial cascade hypothesis has attracted much interest recently [3]. Amyloid cascade hypothesis briefly proposes that, the sufficient accumulation of an amyloid precursor protein (APP) derivative, Aβ, induces the salient biochemical, histologic, and clinical changes which may be manifested in AD patients [4]. Oligomers consisting of the 42 amino acid Aβ derivative ($A\beta_{42}$) are believed to be critical [5]. Other much debatable AD hypotheses (Fig. 1) are: tau hypothesis [6], oxidative stress hypothesis [7], inflammatory hypothesis [8], vascular hypothesis [9], cholesterol hypothesis [10], metal hypothesis [11] and cell cycle hypothesis [12].

AD pharmacotherapy is highly based on modulation of action of neurotransmitters like acetylcholine (ACh), glutamate, serotonin, norepinephrine, dopamine and others [2]. Among these, cholinesterase (ChE) inhibitors play an essential role in the treatment of AD with degeneration of basal forebrain cholinergic neurons; they attenuate the cholinergic deficit, effect positively on cognitive, functional and behaviour symptoms of AD [13]. Two ChE enzymes metabolizing ACh differ in substrate specificity, expression and activity in different brain regions, in health and AD [13,14]. Acetylcholinesterase (AChE, E.C. 3.1.1.7) has catalytic activity at low ACh concentrations and is localized mainly in neurons [13]. It is present in G1 and G4 isoforms in the brain, their abundance vary in different brain areas [15]. Butyrylcholinesterase (BuChE. E.C. 3.1.1.8) is localized mostly in the periphery, only small amount is present in the CNS, especially in regions receiving cholinergic innervation, mainly in glial and endothelial cells [16,17]. When activity and expression of ChE were examined in human aged brain, BuChE was widely distributed compared to AChE activity [15]. Selective loss of AChE G4 isoform was found in AD as well as small increase of G1 isoform of both AChE and BuChE was observed [18]. In AD brain BuChE activity rises, while AChE remains unchanged or declines [19]. Both enzymes therefore represent important targets in AD treatment [16,19].

Up to date, donepezil, rivastigmine and galantamine represent the only ChE inhibitors approved for AD treatment, differing in chemical structures, pharmacologic and pharmacokinetic profiles. The only non-cholinergic alternative used for AD symptomatic treatment is memantine acting as an antagonist of glutamate N-methyl-D-aspartate (NMDA) receptors. The treatment strategies vary and include ChE inhibitors, NMDA receptor modulators, amyloid-β binding dehydrogenase (ABAD, also known as 17β-hydroxysteroid dehydrogenase type 10, 17β-HSD10, HSD10) modulators, monoamine oxidase (MAO) inhibitors, immunotherapeutics, antioxidant agents etc. Nowadays, the approach of AD therapy is based on multiple targeted ligands (Figs. 2 and 3), where ChE inhibition is combined with additional biological properties [20,21]. Attention is devoted to drugs with neuroprotective and disease-modifying properties, candidate molecules positively affect neuronal energy metabolism, mitochondrial functions and have antioxidant properties.

As depicted in Fig. 2, Aβ interacts with plasma membrane and impairs its integrity [22]. In addition, Aβ is transported into the cell and crosses mitochondrial membranes (using translocase of the outer membrane, TOM40, and translocase of inner membrane, TIM23) [23]. Aβ could also interact with mitochondrial proteins from mitochondrial permeability transition pore (mPTP) [24,25], which may in turn affect the mitochondrial membrane potential ($\Delta\psi_m$) [25]. Upon entering into mitochondria, Aβ binds with ABAD, which leads to inhibition of detoxifying role of ABAD [26]. These processes are closely interrelated and can cause mitochondrial dysfunctions, impaired activities of citric acid cycle enzymes (e.g. α-ketoglutarate dehydrogenase, α-KGDH) and complexes of oxidative phosphorylation (OXPHOS), increased production of reactive oxygen species (ROS) and initiation of apoptosis [27,28].

Decreased ATP production leads to impairment of ATP dependent processes and all cellular functions are affected. Decrease of $\Delta\psi_m$ and release of cytochrome *c* (cyt *c*) is followed by mPTPs opening. Cyt *c* and other proapoptotic factors induce the formation of apoptosome, and consequently trigger activation of caspases and apoptosis [29]. Apoptosis inducing factor (AIF) is another proapoptotic factor released by mitochondria. Disengaged AIF is transported into nucleus and triggers caspases-independent apoptosis. Phosphorylated tau (pTau) and Aβ cause enhanced nitrosylation of dynamin-related protein 1 (Drp1) leading to increased mitochondrial fission and neurodegeneration [28]. Further, Aβ inhibits the import of proteins into mitochondria and reduce expression of cyclophilin D (located in the matrix of mitochondria, is a modulatory component of the mPTP). Ability of mitochondria to handle Ca^{2+} is impaired by Aβ and amyloid precursor protein (APP), consequently overload of mitochondrial calcium leads to decrease of $\Delta\psi_m$, opening of mPTP, releasing of proapoptotic factors, increase of ROS production and decrease of ATP production [28].

The enzyme monoamine oxidase (MAO; EC1.4.3.4) is a mitochondrial enzyme, which is located on the outer mitochondrial membrane and catalyses the oxidative deamination of biogenic and xenobiotic monoamines. A major physiological role of

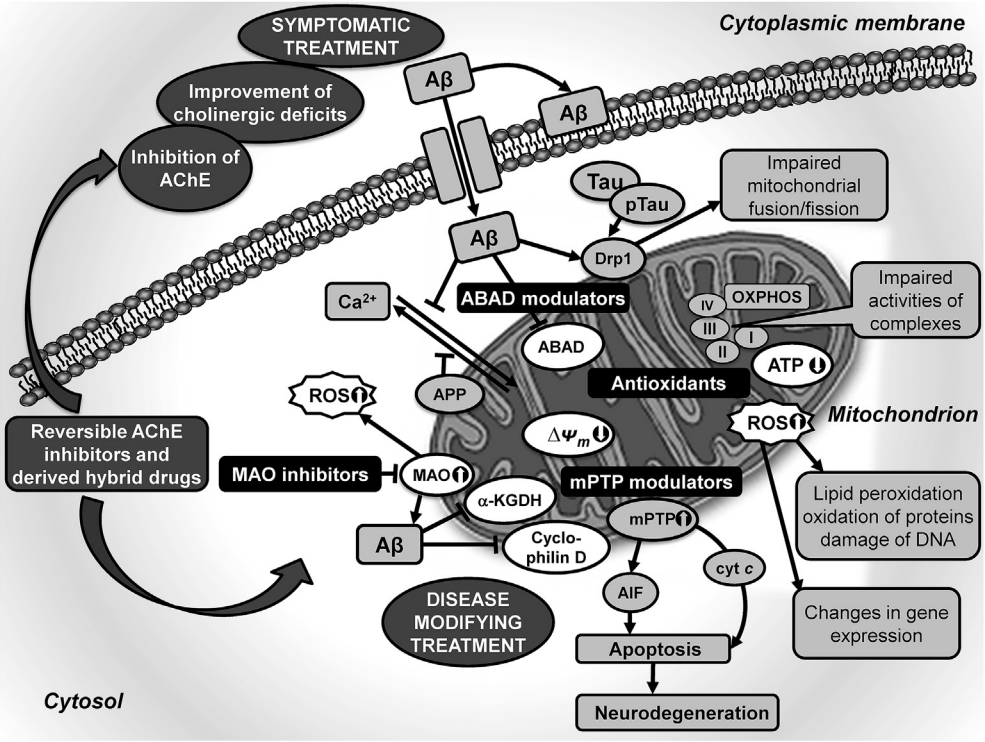

Fig. 2. Mitochondrial dysfunctions in Alzheimer's disease (AD) and pharmacological targets of treatment. Acetylcholinesterase (AChE) represents classical pharmacological target of cholinergic replacement therapy of AD. Inhibition of AChE increase synaptic acetylcholine concentration and improve some symptoms of AD, which are caused by cholinergic deficit. Mitochondrial hypothesis for AD aetiology implicates mitochondrial DNA variation, mitochondrial dysfunction and oxidative stress as one of the primary events in the course of AD. Disease-modifying treatment of AD may be based on modulation of novel non-cholinergic mitochondrial targets, which participate on direct interactions of amyloid-β (Aβ) and/or tau protein with several proteins on and inside mitochondria.

intraneuronal MAO is to keep cytosolic monoamine concentrations very low. MAO exists in two isoforms that differ in substrate preference, inhibitor specificity, tissue and cell distribution, and immunological properties. The type A (MAO-A) metabolizes 5-HT and is sensitive to inhibition by low concentrations of clorgyline, whereas the type B (MAO-B) prefers benzylamine or 2-phenylethylamine (PEA) as substrate and is sensitive to inhibition by low concentrations of l-deprenyl. The high levels of both forms are found in the brain; serotonergic neurons and astrocytes contain predominantly MAO-B. MAO activities were increased in the brains of patients with AD [30,31]. MAO is involved in neurodegeneration via oxidative stress (derived from an increased production of hydrogen peroxide) [32] and increased turnover of monoamine neurotransmitters (such as serotonin, norepinephrine and dopamine) [31]. Activated MAO increases the expression of β-secretase and γ-secretase and improves Aβ generation from APP [31]. Activated MAO could be also associated with the formation of neurofibrillary tangles. Many drugs act as MAO inhibitors. Some of these drugs showed therapeutic value in treatment of depressive or neurodegenerative disorders, including Parkinson's disease and AD [33,34]. Therapeutic effects of MAO inhibitors are related to both lowered catabolism of monoamine neurotransmitters and decreased production of hydrogen peroxide. MAO inhibitors improve cognitive deficits and reverse Aβ pathology by modulating proteolytic cleavage of APP and reducing Aβ. Thus, MAO inhibition is included in mechanisms of action of multitarget drugs, newly developed to treat AD [31].

2. Mitochondrial dysfunctions in AD

Mitochondrial insufficiencies, contributing to pathology of AD, have been described in AD brains, blood cells and fibroblasts [35–37]. At the molecular level, energy metabolism is impaired in AD. Mitochondrial abnormalities and alterations in mitochondrial enzymes, especially in activity of NADH dehydrogenase (ubiquinone) (**complex I**) and cytochrome *c* oxidase (**COX**, complex IV) were observed. Studies reported decreased complex I activity in AD brains [38,39]. There is a connection between currently used AD biomarkers (Aβ, tau protein and phosphorylated tau protein) and mitochondrial dysfunctions [28,40,41], which indicates the role of mitochondrial functions in both pathophysiology of AD and diagnosis of the disease. The inhibition of complex I can be partially explained by the interaction of ND3 (mitochondrial encoded complex I subunit) and Aβ protein [42]. COX deficiency was originally described in AD platelets [43]; later, decreased COX activity was confirmed in mitochondria of AD patients isolated from platelets and from postmortem motor cortex and hippocampus [35]. Degradation of many mitochondrial proteins was reported, including specific citric acid cycle enzymes and **complexes of the electron transport chain** (ETC) [28,35–37,44]. Both, the increased ROS production and disturbed antioxidant protection participate in pathophysiological effects of mitochondrial dysfunctions [45,46].

Nowadays, the extracellular insoluble deposits of Aβ are not supposed to be involved in pathogenesis of AD. Growing evidence support the hypothesis that Aβ oligomers in soluble intracellular forms cause the dysfunction of intracellular organelles [47,48].

Mitochondrial dysfunctions involved in AD pathophysiology include disturbances in OXPHOS, increased mitochondrial DNA (mtDNA) deletions, mutations or polymorphisms, impaired calcium signaling, and impaired energy metabolism as well as interactions with disease specific proteins (e.g. Aβ, tau protein, and α-synuclein) [49]. Aβ induced mitochondrial dysfunction contributes

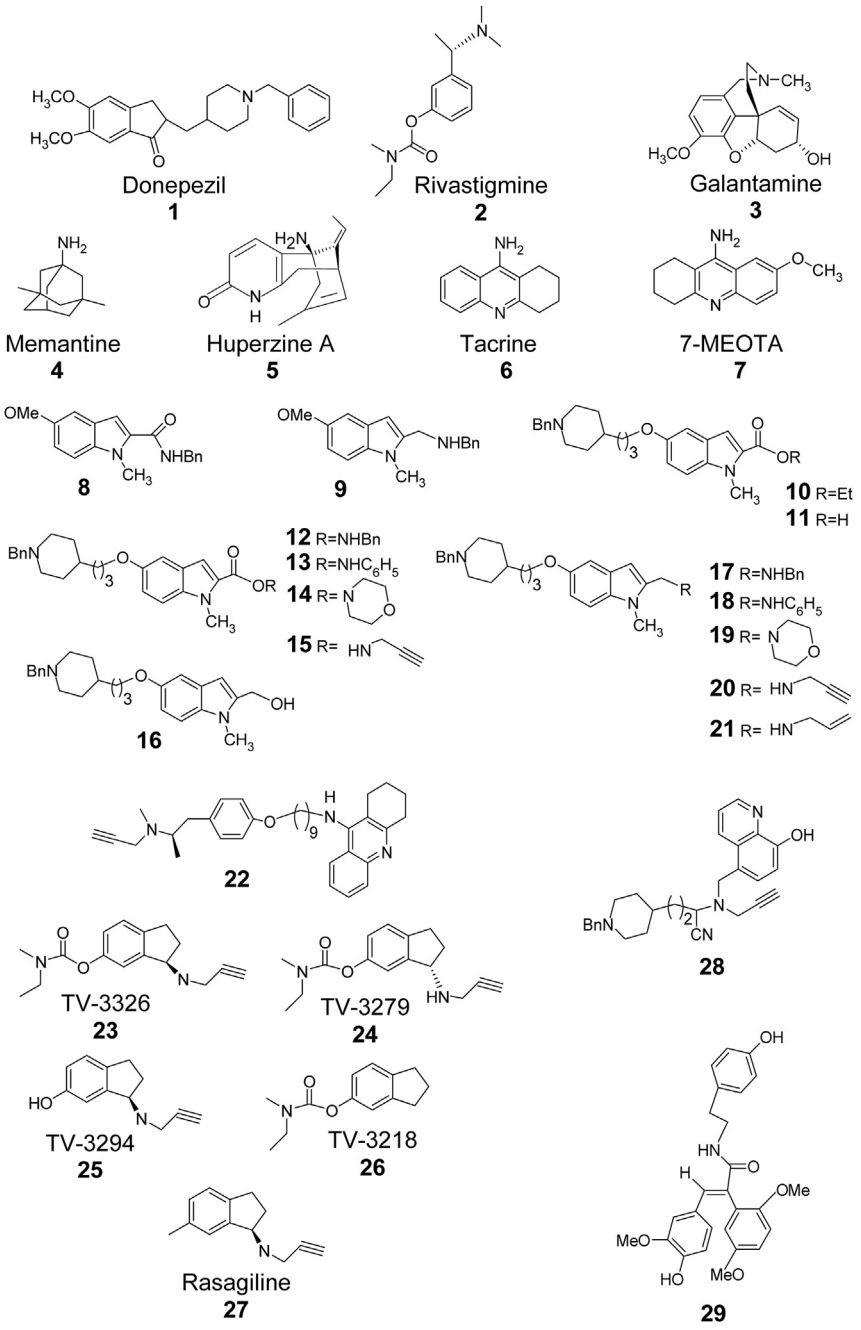

Fig. 3. Panel of structures consists of clinically used drugs and newly developed molecules for AD treatment.

to energy metabolism impairment, defects in key respiratory enzyme activity/function, accumulation/generation of ROS, disruption of $\Delta\psi_m$, altered mitochondrial biogenesis, and dynamics. Mitochondria have intense antioxidant system against ROS and any dysfunction in mitochondrial metabolism leads to increased ROS production ultimately causing neurodegeneration. Despite extensive research efforts to understand the pathophysiology of neurological diseases with respect to mitochondrial dysfunction, the exact mechanism is still not established.

The present review summarizes the current knowledge about the effects of clinically used AD drugs and novel candidates on mitochondrial energy metabolism.

3. Clinically used drugs for treatment of AD

Donepezil (**1**) is a non-competitive and reversible AChE inhibitor. Donepezil attenuated the mitochondrial dysfunctions, decreased mitochondrial calcium and reversed the $\Delta\psi_m$ induced by okadaic acid in rat brain mitochondria [50]. Neuroprotective effect of ChE inhibitors was observed in neuroblastoma SH-SY5Y cells against okadaic acid or $A\beta_{25\text{-}35}$ [51]. U-shaped protective curve was achieved for galantamine (concentration 0.3 μM), for donepezil (1 μM concentration); rivastigmine showed a concentration-dependent effect with the maximum at 3 μM. The blocker of phosphoinositide 3-kinase (PI3K)-Akt reversed the protective effects of galantamine, donepezil, but not the effect of rivastigmine [51]. This mechanism of action is likely not related to AChE

inhibition [51]. Additive protective mechanism of donepezil was related to inhibition of glycogen synthase kinase 3 (GSK-3) and Akt activation [52]. Additionally, effects of donepezil against glutamate neurotoxicity were observed in primary cultures from rat brain cortex; incubation of cortical neurons prevented glutamate-induced apoptosis and consequently apoptotic neuronal death [53].

Donepezil was found to cause the loss of $\Delta\psi_m$, to increase the release of cytochrome *c* to the cytosol, and to alter the expressions of Bcl-2 family proteins [54]. Donepezil displayed an induction of apoptosis in HL-60 cells via a mitochondria-mediated caspase-dependent pathway [54]. Mitochondrial membrane depolarization was significantly visible at 24 h after treatment with donepezil and was further increased at 48 h. The study suggested that donepezil induced the activation of caspase-9 mediated by loss of $\Delta\psi_m$ in HL-60 cells. Loss of $\Delta\psi_m$ is usually associated with the formation of mPTPs and diffusion of cyt *c*, which is normally associated with the inner mitochondrial membrane, into the cytosol. Cytosolic cyt *c* activates procaspase-9 by binding to Apaf-1, which leads to caspase-9 activation and the subsequent activations of downstream executioner caspases (caspases-3, -6 and -7) [54]. Levels of cytosolic cyt *c* were elevated by donepezil in HL-60 cells, which suggested the involvement of the mitochondrial pathway in donepezil-induced apoptosis. These findings suggest that donepezil modulates the protein levels of Bid, Bax, Bcl-2, and Bcl-xl, and that this results in loss of $\Delta\psi_m$ and release of cyt *c* from mitochondria [54].

Rivastigmine (**2**) is a non-competitive pseudo-irreversible inhibitor [55,56]. It differs from tacrine and donepezil in its structure and pharmacokinetic properties [56]; rivastigmine inhibits both AChE and BuChE, with its long inhibition on AChE acting up to 10 h. Rivastigmine was also reported to be responsible for decline of AChE activity in cerebrospinal fluid of AD patients [57–59]. Biochemical studies showed that rivastigmine induces greater selectivity of AChE inhibition in the CNS than in the periphery [56,60]. Therefore, AD patients, deteriorating on selective AChE inhibitor or are unable to tolerate treatment with donepezil, can benefit from a switch to rivastigmine [61,62]. As a dual inhibitor, rivastigmine could provide more sustained efficacy than selective AChE inhibitors and help to slow the formation of amyloidogenic proteins [63].

In degenerating primary rat neurons rivastigmine decreased Aβ secretion and increased α-secretase cleaved secreted APP [64]. Elevated levels of α-secretase cleaved secreted APP could participate in cellular metabolic activity and enhanced neuronal survival [64].

Biochemical analysis performed by Kumar et al. [65] revealed the possible role of rivastigmine against 3-nitropropionic acid (complex II inhibitor) induced behavioural, biochemical and cellular alterations. Further, rivastigmine treatment significantly attenuated oxidative damage and improved mitochondrial complexes enzyme activities in different regions (striatum, cortex and hippocampus) of rat brain. The results show that rivastigmine could be used as an effective therapeutic agent in the management of several neurodegenerative including AD and Huntington's disease.

Rivastigmine exerts a profound effect on lymphocyte mitochondria [66]. A pattern of higher oxidative and enzymatic activities was seen in rivastigmine treated-AD patients when compared with control or untreated-AD. Statistically significant difference was observed for oxidation of pyruvate-malate (substrate of complex I) and glycerol-3-phosphate (substrate of complex III), and for enzymatic activities of complexes II, III and IV. The differences were always present between treated-AD and untreated-AD patients and, in most cases, between treated-AD patients and controls. Rivastigmine enhanced the mitochondrial ability to oxidize substrates for complexes I and III (respiratory capacity), and increased the enzymatic activity of ETC complexes II, III and IV. Although without statistical significance, it stimulated physiological respiration and ability to oxidize succinate, a substrate for complex II. The mechanisms by which rivastigmine would stimulate ETC are uncertain.

Galantamine (**3**) has dual mechanism; it acts as competitive reversible AChE inhibitor and allosterically potentiates nicotinic acetylcholine receptors [67]. Allosteric modulation of nicotinic acetylcholine receptors could have therapeutic benefit in AD [68]. Study using human neuroblastoma SH-SY5Y cells showed increased density of α7 nicotinic receptors and up-regulation of antiapoptotic protein Bcl-2 after the incubation with galantamine [69]. Another *in vitro* study observed that galantamine protected PC12 cells against the Aβ-induced apoptosis. It prevented Aβ aggregation, morphological changes of endoplasmic reticulum and mitochondria, and loss of $\Delta\psi_m$ as well as accumulation of ROS [70]. In human HePG cells galantamine did not induce cytotoxicity, it did not affect oxidative stress markers (increased malondialdehyde production, decreased in glutathione levels), and did not increased ROS production in concentrations lower than 100 μM [71]. Galantamine significantly restored complex I and complex II activity in mice, where the neurodegeneration was induced by intrahippocampal administration of kainic acid [72].

Memantine, (**4**) non-cholinergic alternative to AChE inhibitors, a low affinity uncompetitive antagonist of NMDA receptors has been approved for AD treatment of moderate to severe stage of the disease. Memantine preferentially blocks extensive activity of NMDA receptors without disruption of normal physiological activity, it does not accumulate to interfere with normal synaptic transmission [73,74]. Inhibition of NMDA receptors by memantine prevented the mitochondrial swelling, increased oxidative stress and decreased $\Delta\psi_m$ induced by berberine *in vitro* in primary neurons from mice and rats [75]. Memantine prevented *in vitro* irreversible electrophysiological changes induced by inhibitor of complex II, 3-nitropropionic acid, but it was not effective in protection of complex I, the rotenone toxicity (complex I inhibitor) was not influenced in spiny striatal neurons [76]. Similarly to ChE inhibitors, memantine reduced Aβ levels in neuronal cultures in APP/PS1 transgenic mice [77]. Memantine has also neuroprotective properties and can inhibit Aβ-induced neurodegeneration and enhanced cerebral glucose utilization [67], studies showed decrease in brain glucose metabolism [78]. Memantine attenuated the mitochondrial dysfunctions, reduced mitochondrial calcium, reversed $\Delta\psi_m$ and also reduced the ROS production (donepezil had no effect on ROS levels) induced by okadaic acid in rat brain mitochondria [50]. The effect of memantine was examined on the levels of glial cells, neuropeptides, and peptide-degrading enzymes in rat brain regions of ibotenic acid (IBO)-treated AD model. The study investigated protection effect of co-administration on the changes of neuropeptides, neuronal and glial cells in IBO-infused rat brain by memantine treatment and concluded that glia activation might play an important role to the pathology of AD, and correlate with the changes of neuropeptide levels in AD brain that is recovered by memantine treatment [79]. Arif et al. aimed to investigate the protective effects of memantine on $A\beta_{25-35}$ induced changes in peptidergic and glial systems [80]. Treatment with memantine appreciably increased $A\beta_{25-35}$ induced changes of neuropeptides, their metabolizing enzymes and glial marker proteins. They suggested that memantine exerts its protective effects by modulating the neuropeptide system as a consequence of suppressing the glial cells and oxidative stress in AD model rat brain. The memantine protection against Aβ induced neurotoxicity and learning impairment was reported in rats [81]. They concluded that memantine, at therapeutically relevant concentrations, can protect against neuronal degeneration induced by Aβ. The probable role of memantine against Aβ-induced toxicity; memantine treatment

remarkably protected cultured neurons against Aβ-induced toxicity by attenuating tau-phosphorylation and its related signaling mechanisms [82]. However, this drug did not alter either conformation or internalization of $A\beta_{1-42}$ and it was unable to attenuate Aβ-induced potentiation of extracellular glutamate levels.

4. ChE inhibitors in preclinical studies

Huperzine A, (**5**) an active alkaloid from Lycopodium, was described as a selective and reversible AChE and BuChE inhibitor, with neuroprotective effects against the glutamate induced toxicity and anti-inflammatory properties [83,84]. Interaction with excitatory amino acid neurotransmitter system and subsequent glutamate-induced calcium mobilization was observed after the incubation of neuronal cultures with huperzine A, reduction of NMDA mediated toxicity has been found. This blockage of NMDA ion channels without any psychomimetic effects could be useful in diverse neurodegenerative diseases [85]. Next to these effects, huperzine A has been described as disease modifying drug with neuroprotective effects directly acting on mitochondria [86]. It ameliorated the effect of $A\beta_{1-42}$ on ATP reduction and mitochondrial swelling, as well as a decrease in the enzymatic activities of respiratory chain complexes (complex II/III and complex IV) in isolated brain mitochondria from double transgenic AβPP/PS1 mice [87]. In APP/PS1 double-transgenic mice and SH-SY5Y cells huperzine A inhibited GSK-3β and increased the level of β-catenin. These findings suggest that the neuroprotective effect of huperzine A can be related to the targeting of the Wnt/β-catenin signaling pathway [88].

Compared with tacrine, donepezil, and rivastigmin, huperzine A has better penetration through the blood–brain barrier, higher oral bioavailability, and longer duration of AChE inhibitory action and fewer peripheral cholinergic side effects [89]. Huperzine A possesses the ability to protect cells against hydrogen peroxide, Aβ, glutamate, ischemia, staurosporine-induced cytotoxicity and apoptosis [89].

It is worth noting, that the mitochondrial Aβ levels are associated with the extent of mitochondrial dysfunction in different brain regions and the degree of cognitive impairment in AD; e.g. Aβ increases the neuron vulnerability to oxidative stress and impairments of electron transport chain [90]. Accumulation of Aβ and α-synuclein oligomers in the mitochondrial membrane might result in the release of cyt *c* with the subsequent activation of the apoptosis. Conversely, the oxidative stress and mitochondrial dysfunction associated with AD may lead to increased membrane permeability and cyt *c* release, which promotes Aβ and α-synuclein oligomerization and neurodegeneration [22].

Donepezil, galantamine as well as huperzine A increased the viability of neurons against $A\beta_{42}$ toxicity [52]. On cellular level, donepezil reduced calcium-induced mitochondrial swelling in APP/PS1 transgenic mice [48].

4.1. Tacrine derivatives

Tacrine (**6**) was the first registered reversible ChE inhibitor. It was withdrawn from the market due to its poor selectivity towards AChE, which resulted in a number of adverse effects (hepatotoxicity, gastrointestinal discomfort) [91]. Recently, novel tacrine and **7-methoxytacrine** (7-MEOTA, **7**) **derivatives** were synthesized and extensively investigated to find less toxic compounds affecting more AD pathological mechanisms. 7-MEOTA derivatives have lower toxicity while retaining pharmacological properties of tacrine and are promising candidates for AD treatment [92]. Their ability to inhibit AChE and BuChE were evaluated on recombinant human AChE and plasmatic human BuChE. An effort from our group [93] was to examine the effect of ChE inhibitors (tacrine, 7-methoxytacrine) on the activity of complex I in brain mitochondria. Inhibition of complex I by tacrine was statistically significant, which suggested the possibility of tacrine-induced side effects related to disturbances of ETC. Further *in vitro* testing is necessary for evaluation of new tacrine derivatives as candidate molecules for the treatment of AD.

4.2. Synthesized hybrid cholinesterase inhibitors

The multifactorial and complex nature of AD makes inadequate the use of magic bullets targeted to a single receptor or enzymatic system for the efficient treatment of the disease. However, it is now widely accepted that a more effective therapy would result from the use of multipotent compounds able to intervene in the different pathological events underlying the aetiology of AD [20].

4.3. Tacrine-derived hybrid drugs

Additionally to tacrine and 7-MEOTA, tacrine-flavonoids, tacrine-coumarins, tacrine-trolox hybrids and tacrine-propargylamine derivatives have been recently designed, synthesized and evaluated as multifunctional ChE inhibitors [94–99]. Tacrine-propargyline derivatives exhibited balanced AChE and BuChE activities, increased human AChE activity, whereas lower neurotoxicity and hepatotoxicity compared to tacrine [96].

Tacrine-coumarin hybrids exhibited an ability to inhibit ChE and induced self-Aβ-aggregation, they acted also as metal chelators [99]. A new series of tacrine-flavonoid hybrids were designed and synthesized, most of the compounds inhibited both AChE and BuChE activities [95]. A tacrine-flavonoid hybrid was found to be a potent and balanced inhibitor against ChE, and induced self-aggregation with $A\beta_{1-42}$ [95]. Combining tacrine with trolox, a strong antioxidant, resulted in synthesis of molecule, which was less hepatotoxic than tacrine, strong inhibitor of AChE and BuChE showing neuroprotective effects [98]. Another designed tacrine hybrid was presented with the ability to inhibit AChE and BuChE, good inhibition of Aβ aggregation and good antioxidant activity [94]. Phenylthiazole-tacrine hybrids were examined as cholinesterase inhibitors, blocking $A\beta_{1-42}$ aggregation and calcium overload [97].

4.4. Donepezil-derived hybrid drugs

Donepezil-indolyl hybrids (**8**–**21**) were designed (see Fig. 4), synthesized and pharmacologically evaluated as multipotent ASS234 analogues (amines, amides, carboxylic acids), which are able to inhibit simultaneously cholinesterase (ChE) and MAO

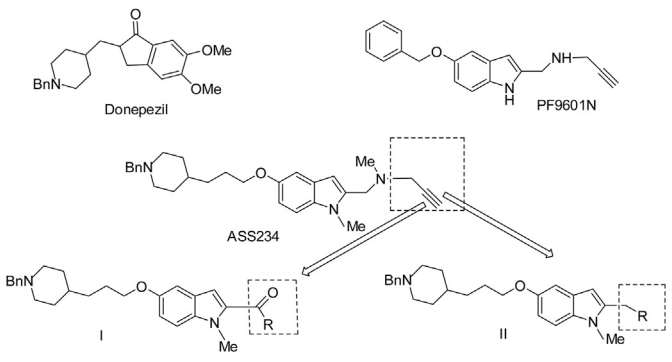

Fig. 4. General structure of donepezil, ASS234, PFN601N and novel MAOI/ChE hybrids I and II.

enzymes [100]. By performing *in vitro* analysis, it was concluded that the amines are in general more potent ChE inhibitors (see compounds **17**, **18** versus **12** and **13**) or equipotent (see compounds **19**, **20** versus **14** and **15**) than the corresponding amides. Amides were not active in inhibition of MAO; among the amines, compounds **19**, **20** and **21** were MAO-A selective. Carboxylic acid derivatives **10** and **16** showed a multipotent moderate selective profile as AChE and MAO-A inhibitors. As a result N-(5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl) prop-2-yn-1-amine (**15**),was identified as a potent inhibitor of MAO-A (IC_{50}, 5.5 ± 1.4 nM), moderately potent inhibitor of MAO-B (IC_{50}, 150 ± 31 nM), and inhibitor of both AChE (IC_{50}, 190 ± 10 nM), and BuChE (IC_{50}, 830 ± 160 nM) [100].

5. Inhibitors of MAO-B and derived hybrid drugs

Propargyline derivatives, selegiline and rasagiline, are selective irreversible MAO-B inhibitors used in Parkinson's disease treatment. High expression levels of MAO-B can result in increased level of ROS and might play a role in etiology of AD.

Selegiline has antioxidant and neuroprotective effects; it reduced lipid peroxidation in prefrontal cortex, striatum and hippocampus and increased the activity of glutathione peroxidase activity; these effects can attenuate the neurodegenerative processes in aged rats [101,102]. New carbamate derivatives of aminoindans (rasagiline-related series) and phenylethylamines (selegiline-related series) have been described as dual inhibitors of AChE and MAO [103]. Novel compounds were designed using a combination of benzylpiperidine moiety of donepezil and indolylpropargylamino moiety of MAO inhibitor [104].

A series of tacrine-selegiline hybrids was synthesised and evaluated as inhibitors of cholinesterase (AChE/BuChE) and MAO-A/B [105]. The results demonstrated that most of the synthesised compounds exhibit high inhibitory activity. Among these compounds, compound **22** provided a good balance of activity towards all targeted enzymes: AChE, BuChE, MAO-A and MAO-B, respectively. These results indicated that **22** has the potential to be a multi-functional candidate for AD.

The novel drug ladostigil tartrate (TV-3326, **23**) is derived from a combination of two pharmacophores: the carbamate moiety from rivastigmine, an AChE inhibitor, and propargyl group from rasagiline, a MAO-B inhibitor. This drug exhibits both cholinesterase and selective MAO inhibitory activities in the brain, reduces apoptosis and stimulates the processing of APP, hence reducing the possibility of generating the toxic Aβ [106].

A series of novel propargylaminoindans with a carbamate moiety to inhibit cholinesterase were developed from phamacophore of rasagiline to protect or rescue deteriorated neurons in AD and Lewy body disease and provide a beneficial effect on the cognitive deficits [107]. The carbamate moiety of rivastigmine was introduced into the 6 position of the rasagiline molecule to provide ChE inhibitory activity [108–110]. Rasagiline analogues were found to protect dopaminergic SH-SY5Y cells against apoptosis induced by peroxynitrite donor, SIN-1. TV3326, [(N-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate, was as effective as rasagiline in preventing apoptosis, followed by its S-enantiomer, TV3279 (**24**). The anti-apoptotic-neuroprotective activity was shown to reside in the propargylamine and not the carbamate moiety. This resulted in stabilization of the $\Delta\psi_m$, the collapse of which initiates the apoptotic cascade.

Neuroprotective activities of TV3326, its S isomer, TV3279, and related compounds were examined for their potential protection against apoptosis and the fall in $\Delta\psi_m$ associated with apoptosis induced by the peroxynitrite-generating agent, SIN-1 [N-morpholinosydnonimine] in dopaminergic neuroblastoma SH-SY5Y cells [107]. The presence of the carbamate moiety in TV3326 and TV3279, did not affect the anti-apoptotic function associated with rasagiline. The metabolite TV3218 (**26**), devoid of propargyl moiety, was devoid of anti-apoptotic activity. By contrast the hydroxylpropargyl major metabolite of TV3326, TV3294 (**25**), had anti apoptotic activity similar to rasagiline (**27**) and TV3326 and TV3279.

The neuroprotective effects of tacrine hybrids could be associated with the inhibition of AChE induced Aβ aggregation and inhibition of β-secretase. Two isomeric series of dual binding site acetylcholinesterase inhibitors have been designed and synthesized by Camps et al. [111]. These hybrids, consisting of a unit of 6-chlorotacrine and pyrano [3,2-c]quinoline, possessed the potent and selective human AChE inhibitory activity and exhibited a significant *in vitro* inhibitory activity toward the AChE-induced and self-induced Aβ aggregation and toward β-secretase, in addition to the ability to enter the central nervous system [112].

Donepezil + propargylamine + 8-hydroxyquinoline hybrids (DPH, **28**) are multifunctional metal-chelators, ChE and MAO inhibitors. They have been newly synthesized and biochemically evaluated for potential AD treatment investigated [113]. The most interesting derivative was racemic derivativeα-aminotrile-4-(1-benzylpiperidin-4-yl)-2-(8-hydroxyquinolin-5-yl)methyl) (prop-2-yn-1-yl)amino)butanenitrile (DPH6). It was characterized as irreversible MAO-A/B inhibitor and mixed-type AChE inhibitor with metal-chelating properties [MAO-A (IC_{50} = 6.2 ± 0.7 μM; MAO-B (IC_{50} = 10.2 ± 0.9 μM); AChE (IC_{50} = 1.8 ± 0.1 μM); BuChE (IC_{50} = 1.6 ± 0.25 μM)]. These DPH hybrids performed good blood brain barrier penetration and were found to be less toxic in an *in vitro* tested toxicity in HepG2 cells [113].

Liu et al. [114] have investigated the effects of N-[2-(4-hydroxyphenyl)ethyl]-2-(2,5-dimethoxyphenyl)-3-(3-methoxy-4-hydroxyphenyl)acrylamide, compound FLZ (**29**), a novel synthetic analogue of squamosamide, on the dysfunction of rat brain mitochondria induced by $A\beta_{25-35}$ *in vitro*. The activities of mitochondrial enzymes, production of hydrogen peroxide and superoxide anion and the levels of glutathione in mitochondria were examined. Incubation of mitochondria with aged $A\beta_{25-35}$ inhibited the activities of α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and complex IV of electron transport chain. Increased hydrogen peroxide and superoxide anion production, and decreased the glutathione level were observed. Furthermore, it induced mitochondrial swelling and cyt *c* release from the mitochondria. The addition of FLZ (100 μmol/L) prior to treatment with $A\beta_{25-35}$ significantly prevented these toxic effects of $A\beta_{25-35}$ on rat brain mitochondria and *in vitro* protective effects of FLZ were concluded. Another study examined the effects of FLZ in APP-SH-SY5Y cells, FLZ selectively inhibited γ-secretase and decreased accumulation of Aβ in mitochondria [115]. The inhibitory effect of FLZ in APP/PS1 double transgenic mice and SH-SY5Y cells on GSK-3β activity and tau phosphorylation was observed. FLZ inhibited also Akt activity, which indicated that Akt/GSK-3β pathway might be the possible mechanism of involved in the inhibitory effect of FLZ on tau hyperphosphorylation. These results suggested FLZ as potential for AD therapy, reducing Aβ production via both mechanisms inhibition amyloidogenic APP processing pathway and attenuated tau hyperphosphoylation mediated by Akt/GSK-3β [116].

6. Drugs interacting with mitochondrial enzymes

Aβ forms extracellular plaques, in the cell Aβ interacts with intracellular targets such as mitochondrial proteins, ABAD and cyclophilin D (CypD) [117]. ABAD is dehydrogenase interacting with Aβ and promoting Aβ-mediated mitochondrial and neuronal dysfunctions [118]. Aβ-ABAD complex was examined by high-

resolution crystallography, deformation of ABAD structure with exclusion of NAD^+ cofactor was demonstrated in the Aβ presence [24]. ABAD enhanced Aβ-induced cell stress via decreased COX activity and exacerbated leakage of ROS in neurons, these changes were found in cultured neurons from transgenic mAPP/ABAD mice. Therefore, design of **ABAD modulators** and targeting of mitochondrial ABAD represent a novel strategy of AD treatment [118,119]. Firstly, synthesized ABAD modulators (ABAD-4a, ABAD-4b) increased the COX activity and ATP levels and suggested the protective effects on mitochondrial properties [118]. Frentizole, immunosuppressive drug, was identified as inhibitor of Aβ-ABAD interaction; other benzothiazole urea derivatives and frentizole analogues have been developed [120].

The role of CypD-dependent mPTP was reported in Aβ-impaired axonal mitochondrial trafficking [121]. Depletion of CypD protects axonal mitochondrial motility and dynamics from Aβ toxicity as revealed by augmented axonal mitochondrial density and distribution and improved bidirectional transport of axonal mitochondria. Notably, blockade of mPTP by genetic deletion of CypD suppresses Aβ-mediated activation of the p38 mitogen-activated protein kinase signaling pathway, reverses axonal mitochondrial abnormalities, recovers synaptic function, and reduces loss of synapse, suggesting a role of CypD-dependent signaling in Aβ-induced alterations in axonal mitochondrial trafficking. Very interesting review summarizing the progress on mPTP and its potential therapeutic target for neurodegenerative diseases including AD was presented by Rao [121]. The authors have also reported that interaction of CypD with mitochondrial Aβ potentiates mitochondrial, neuronal and synaptic stress [122]. Their findings have manifested that the CypD and Aβ directly interact with each other in the mitochondria of AD brain and in a transgenic mouse model of AD. According to their study, CypD-Aβ interaction promotes ROS generation and CypD recruitment to the mitochondrial inner membrane, leading to the formation of the mPTP. The CypD-deficient cortical mitochondria are resilient to Aβ- and Ca^{2+}-induced mitochondrial swelling and permeability transition. They have better calcium buffering capacity and generate fewer mitochondrial reactive oxygen species. The absence of CypD protects neurons from Aβ- and oxidative stress-induced cell death. Notably, CypD deficiency significantly develops learning and memory and synaptic function in an AD mouse model and improves Aβ-mediated reduction of long-term potentiation. Thus, the CypD-mediated mPTP is directly linked to the cellular and synaptic perturbations observed in the pathogenesis of AD.

Novel series of quinazoline-urea derivatives as modulators of Aβ induced mitochondrial dysfunctions were prepared and studied [123]. Their blocking activities against Aβ induced disruption of $\Delta\psi_m$ and led to mPTP. From the results the active nonpeptidyl mPTP blockers can be considered as a new direction for the design of novel **mPTP modulators**.

7. Conclusions

The current review deals with development of drugs for AD in relation to energy metabolism and involvement of other possible mechanisms of AD drugs. Significant achievements with clinically approved drugs and promising synthesized candidates under trials have been discussed in detail. Through the current report we have tried to gain an insight into the mitochondrial functions: OXPHOS, individual mitochondrial enzyme activities, effects on ROS production and $\Delta\psi_m$, calcium homeostasis, antioxidant properties and MAO-B activity and interference of drugs with these functions. The present review may contribute to the efforts being made to understand the role of mitochondria in the pathophysiology of neurodegeneration and may eventually provide new therapeutic strategies for neurodegenerative diseases.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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