ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 3, 13 SECTIO DDD 2010

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Poland

KINGA TENDERA, MARTA KRUK, GRAŻYNA BIAŁA

# *Alzheimer's disease: causes, symptoms and pharmacotherapy*

Choroba Alzheimera: przyczyny, objawy i farmakoterapia

## INTRODUCTION

How important is memory? The study of memory can usefully investigate many aspects of pharmacological analysis. Many important issues are under discussion, including the elucidation of what memory is and which brain systems are involved in these processes. It should be noted that memories in the brain cannot be directly observed. Their presence must be inferred from clear behavioral expression. Memory, as measured by changes in an animal's behavior some time after learning, reflects many processes including acquisition, consolidation, retention, retrieval and performance. Knowledge of these processes of memory can be helpful to investigate learning and memory functions in safety pharmacology and can provide new perspectives for promising therapy in the treatment of human memory impairment like Alzheimer's disease [8].

## PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a multifactorial, progressive, terminal neurodegenerative brain disorder and the most common form of dementia characterized by impaired cognitive functions. The disease was first described by doctor Alois Alzheimer, who diagnosed it in his patient – a fifty-year-old woman Auguste D. Nowadays the prevalence of Alzheimer's disease is 10.8% cases in people aged 80–90 years, although the early-onset of Alzheimer's can also occur (0.02% in people aged 30–39 years) [2]. The Alzheimer's Association estimated that in 2007 in the United States of America more than 5 million people have AD. The number of cases of AD is increasing and probably by 2050 is expected to amount approximately to 13 million persons [12].

The earliest symptoms of disease are frequently missed by the family and incorrectly thought to be age-related. First, AD leads to small deficits in cognitive functions, which become bigger during the progress of dementia. The most commonly recognized symptoms of AD in the early stages of neurodegeneration are inability, or difficulty to recollect facts, information and memory deterioration or loss (amnesia). Other symptoms of AD are difficulties in producing or comprehending spoken or written language (aphasia), impairment of the ability to carry out learned purposeful movements, such as writing, playing an instrument

(apraxia) and loss of the ability to recognize known objects or persons (agnosia). Second, in consort with neurodegeneration some behavioral disturbances and psychiatric symptoms manifest, such as personality change, mood swings, depression, misidentifications, irritability and aggression [2].

The most popular theory explaining the neuronal degeneration in AD is the amyloid hypothesis. β-amyloid is a piece from bigger protein termed amyloid precursor protein (APP). APP is very important to neuron increment, survival and repair [13]. In Alzheimer's disease APP become divided into smaller parts by enzymes β-secretase and γ-secretase through proteolysis generating β-amyloid, which form aggregates that deposit outside neurons [12]. According to amyloid hypothesis, the production and accumulation of β-amyloid peptides derived from the enzymatic processing of APP initiate the neuronal death. *β*-Amyloid plaques (also termed senile plaques) develop between neurons and cause inflammation, apoptotic cell death cascade activation, synaptic degeneration, demyelization, oxidative stress, neurotransmitter deficits and destruction of neighboring neurons [12]. In comparison to normally aging brain, in the brain of people suffering from AD a higher concentration of β-amyloid$_{42}$ is observed (it is the toxic form of amyloid containing 42 amino acids). β-amyloid peptides containing 1–42 amino acids are considered to have the biggest tendency to form into aggregates and to be more hydrophobic and neurotoxic than shorter peptides. However, the up-to-date data suggest that the most neurotoxic form of β-amyloid is its intracellular and extracellular oligomers, rather than, as it was thought, aggregates. Recent studies also suggest that the level of amyloid oligomers positively correlate with the memory impairment in patients with AD. Other studies report that β-amyloid oligomers impair the synaptic plasticity in the brain and induce synaptic declines. The trimmers are believed to be the most toxic oligomeric forms of amyloid [4].

Apart from senile plaques typical microscopic changes occurring in people suffering from AD are neurofibrillary tangles (NFT). NFT are pathological aggregates of protein which develop inside neurons. They are formed by protein termed tau (τ) after its hyperphosphorylation (protein becomes insoluble). Both amyloid plaques and neurofibrillary tangles are the histopathological hallmarks of AD [3, 12].

AD is characterized by the loss of neurons in brain. Deterioration in neuronal pathways involves neurotransmitters, especially acetylcholine – the transmitter essential to the cognitive function. Substantial degeneration of cholinergic neurons in the basal forebrain and a decrease in activity of choline acetyltransferase have been identified by scientists [10].

Knowledge about the pathophysiology of AD is pivotal to develop potential AD-modifying therapies (Fig. 1).

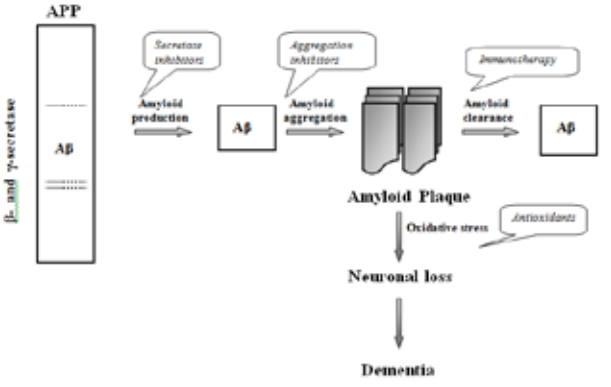


Fig. 1. Selected drug targets in pharmacotherapy of Alzheimer's disease

## PHARMACOTHERAPY OF ALZHEIMER'S DISEASE

### CHOLINESTERASE INHIBITORS

Cholinesterase is an enzyme which breaks down the neurotransmitter acetylcholine. Data showed that there is a relationship between the level of synaptic acetylcholine and cognitive functions improvement. Inhibition of the activity of cholinesterase leads to an increased level of acetylcholine in the brain and has positive effects on the symptoms of AD.

Cholinesterase inhibitors (AChEI) are the most popular group of drugs already registered and applied in USA and/or in Europe to treat AD. Various drugs from the group AChEI (e.g. donepezil, galanthamine, metrifonate, rivastigmine, tacrine) can cause more or less therapeutic effects measured by cognitive improvement, which depends on their level of inhibition of the enzyme cholinesterase. The major effect of AChEI is to maintain the brain cognitive functions in patients with AD at a constant level during 6 months to one year of treatment in comparison to placebo. Moreover, drugs from this group improve daily living conditions [5] and reduce some of the neuropsychiatric manifestations of AD (irritability, apathy, anxiety, paranoia) [7]. The research also suggest that AChEI and agonists of muscarinic receptors can increase the release of non-amyloidogenic and soluble derivatives of APP both *in vitro* and *in vivo* and arguably retard the formation of amyloidogenic complexes in human brain [5].

### APPROACHES MODULATING SECRETASES ACTIVITY

The process of β-amyloid formation from APP in human brain is mediated by two enzymes: β-secretase and γ-secretase, while the cleavage of APP initiated by α-secretase leads to soluble products. The inhibitors of β- and γ-secretase are potential disease-modifying drugs in Alzheimer's disease. There are researches showing that β-secretase knockout mice produce less β-amyloid and that inhibitors of β-secretase injected into the hippocampus significantly reduce the production of β-amyloid *in vivo*. Reduction of β-amyloid concentration in rodent's brain was also observed after the administration of γ-secretase inhibitors. The treatment of Alzheimer's disease also includes attempts to use γ-secretase modulators, which modulates enzyme to produce less of the $\beta\text{-amyloid}_{42}$ and more of the shorter peptides (nontoxic forms). These drugs are shown to reduce the generation of β-amyloid by human cells [12].

### ANTI-INFLAMMATORY DRUGS

Inflammation plays an important role in AD pathogenesis. The inflammatory reaction is supposed to be chronic in patients with AD. Probably, the insoluble, pathologic aggregates of amyloid lead to activation of inflammatory reaction cells (especially microglial cells and astocytes), because they are recognized as a foreign material. The data showed that long-lasting usage of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) noticeably reduces the risk of AD. It is believed that neuronal cyclooxygenase-2 (COX-2) takes part in progression of neurodegenerative processes in AD. It is

observed that transgenic mice with overexpression of COX-2 suffer from age-related cognitive and memory disorders associated with astrocytes activation and neuron's apoptosis in their brain. Moreover, the *post mortem* examinations prove the effectiveness of NSAIDs in decreasing inflammation processes in patients with AD. The mechanism of the protective activity of NSAIDs is still unknown. It is possible that except inhibiting COX activity there is another mechanism (for example, inhibition of β- or γ-secretase) explaining their effectiveness in AD. It is also found that some of the NSAIDs act directly on β-amyloid production (ibuprofen, flurbiprofen, indomethacin and sulindac induce even a 80% decrease of β-amyloid levels in transgenic animal brain). However, further research to prove the effectiveness of NSAIDs in AD is necessary [4].

## IMMUNOTHERAPY

A modern and very promising approach for the AD treatment is immunotherapy including vaccination and immunization. The number of data suggesting that immunotherapy can be effective in human patients suffering from AD is rapidly growing. There are two strategies of immunotherapy. First, termed active immunization is based on usage of β-amyloid or β-amyloid fragments. The second one termed passive immunization use prepared anti-β-amyloid antibodies. The therapeutic potential of producing or delivering antibodies consists in recognizing β-amyloid by antibodies and attenuating amyloid aggregation-associated pathologies. Both active and passive immunization was assessed in mouse models of Alzheimer's disease and results are auspicious; however, passive immunization is considered to be a safer strategy than active vaccination with β-amyloid. Currently, some β-amyloid-selective monoclonal antibodies are being tested. Results of studies on the efficiency of AD-related immunotherapy show reduced brain amyloid plaques, restored neuron and synapse functioning, reduced astrocytosis and improved behaviour. However, further studies are needed to eliminate some immunotherapy-related risks like: autoimmune diseases, brain inflammation or blood brain barrier passage of antibodies [12].

## NATURAL ANTIOXIDANTS

It is known that neurodegenerative diseases such as AD are associated with oxidative stress which induces neuronal apoptosis. Scientists proved that β-amyloid produces hydrogen peroxide and participates in processes which are probably related to yhe production of free radicals. Free radicals, not only peroxidize membrane lipids leading to cell destruction, but also oxidize proteins and damage nucleic acids DNA and RNA. The brain tissue is very sensitive to prooxidant-antioxidant homeostasis and high oxidative stress occurs in degeneration of brain neurons. Antioxidants such as flavonoids, polyphenols and some vitamins should have therapeutic effects on AD because they can cure the disease by protecting neurons preventing and eliminating oxidative stress [14].

F l a v o n o i d s are natural antioxidants isolated from plants. It is investigated that many of flavonoids protect rat cells and neurons from oxidative injuries like for example glutamate toxicity. There are three structural requirements of flavonoids to be effective in the protection from glutamate: unsaturated C ring, hydroxylated C3 and hydrophobicity. A representative antioxidant is Ginkgo Biloba extract. Extracts from Ginkgo Biloba leaves contain flavonoids and also ginkgolides and bilobalides.

These extracts have been reported to have brain-protecting properties by inhibiting reactive oxygen production in neurons, reducing hypoxic damage and protecting cells against apoptosis [14].

Green tea polyphenols are also reported to have potent antioxidative properties. The major constituent of Green tea salubrious polyphenols complex is epigallocatechin gallate (EGCC) which has a lot of beneficial pharmacological activities such as antimutagenic and anticarcinogenic effects. Green tea polyphenols are demonstrated to have powerful antioxidant activity against free radicals like diphenylpicrylhydrazyl radicals (DPPH), superoxide anion, hydroxyl radicals and lipid free radicals. Scientists have also found that transgenic mice overproducing β-amyloid treated EGCC have decreased β-amyloid levels and reduced amyloid plaques, which suggests that EGCC reduce amyloid generation. These findings imply the possibility of Green tea polyphenols supplementation in people suffering from AD as efficient prophylaxis [14].

There are also studies suggesting that some vitamins have important antioxidant functions and can help to protect neuron cells against oxidative stress and damage associated with neurodegenerative diseases such as AD. Very powerful antioxidants are ***vitamin C (ascorbate)*** and ***vitamin E (tocopherol)***. Decreased levels of these vitamins lead to increased oxidative stress. In one study scientists have shown that vitamin C administered intraperitoneally before testing mice in the Morris water maze test, partially attenuated cognitive deficits (amnesia) induced by scopolamine in young mice. Vitamin C also increased acetylcholinesterase activity in the medial forebrain area, which suggests that it may be connected with cholinergic signaling [6]. Results of another study verifying the role of Vitamins C and E on the cognitive functions in mice indicate a considerable improvement in the cognitive functions of aged animals but there were no significant results in young mice [1]. However, further investigation to prove the cognition-enhancing effects of vitamin C and vitamin E is needed.

## CAFFEINE

Caffeine is a natural chemical compound (xanthine alkaloid) of some plants widely consumed by people in the world as coffee, tea, soft or energy drinks. Caffeine is recognized as a central nervous system stimulant which acts mainly by competitive inhibition of adenosine receptors in brain. After binding to adenosine receptors caffeine leads to the elevation of acetylcholine and serotonin levels – neurotransmitters involved in memory functioning. Moreover, caffeine intake affects an increased number of adenosine receptors in animals brain. However, for this study the most significant consequences of adenosine antagonism is the stimulation of cholinergic system, which might lead to the improvement of cognitive functions, especially concentration and memory. There are studies indicating the relation between the human cognitive performance and habitual caffeine consumption. The chronic caffeine intake was noticeably related to improved memory, especially the long-term memory compared to placebo. Moreover, low doses of long-lasting administered caffeine are considered to improve psychomotor performance and they have neuroprotective effects. These neuroprotective properties of caffeine were analyzed in some experimental models of ischemia and hypoxia and are believed to be protective in patients with AD [9].

## NICOTINE

It has been reported that nicotine, a natural alkaloid found in plants from the family *Solanaceae,* is a substance which improves cognitive functions and memory by reaction with nicotinic cholinergic receptors and intensifying the cholinergic transmission. Nicotine may improve cognitive functions by direct stimulation of nicotinic cholinergic receptors or by releasing acetylcholine, glutamate, serotonin, dopamine and other neurotransmitters connected with learning and memory after interaction with presynaptic receptors [11]. Nicotine and nicotinic receptors agonists are considered to have a profitable influence on cognitive functions and memory in AD. The underlying mechanisms of neuroprotective activity of nicotine are still unclear. New findings report that nicotine has a scavenging effect on free radicals (on hydroxyl radicals and superoxide radicals even higher than vitamin C) studied by electron spin resonance techniques. These findings suggest nicotine to be a potential antioxidant. Nicotine was also found to decrease β-amyloid aggregation in unclear mechanism probably associated with regulation of metal homeostasis by nicotine [14].

There are also a lot of data which shown that there are other multiple agents which may be helpful in pharmacotherapy of AD:

## MISCELLANEOUS AGENTS

Statins are inhibitors of enzyme HMG-CoA reductase and a class of drugs used to lower the cholesterol level in plasma in the treatment of dyslipidemia. Results of some research suggest that in patients using statins a considerably reduced risk of AD has been observed. The positive effect of statins in AD might be related to the decrease of β-amyloid production by increasing the γ-secretase pathway of APP processing not by their cholesterol-level-lowering properties [12].

Agonists of M1 muscarinic receptors in some research show the activity to increase the non-amyloidogenic pathway of APP processing and to decrease the levels of β-amyloid both *in vitro* and *in vivo*. M1 muscarinic agonists are probably connected with some mechanisms related to AD including hyperphoshorylation of tau protein, β-amyloid generation and cholinergic functions [12].

Antagonists of NMDA receptors are believed to be effective in AD treatment because some processes (β-amyloid production, overexpression of τ protein, brain damage from excitotoxicity and neuronal cell death) are thought to be connected with overactivation of NMDA receptors. Already approved for AD treatment, NMDA antagonist is memantine. In comparison to placebo, memantine was beneficial in patients with AD in 73% versus 45% of patients getting placebo [7,12].

## CONCLUSIONS

Today there is no effective treatment of AD which can stop the disease progression. However, there are numerous trials of application of various therapies or drugs in AD with different mechanisms of action and molecular targets. Some applied drugs may help keep AD symptoms from getting worse (e.g. AChEI, natural antioxidants, anti-inflammatory drugs). The main purpose for scientists

is to find an effective drug to stop neurodegeneration or prevent the pathological changes in neurons. The most promising in this context seem to be approaches modifying the activity of secretases and immunotherapy.

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## SUMMARY

AD is the most common form of dementia characterized by a noticeable decline in cognitive functions. There are several theories explaining the neuronal degeneration in AD including the β-amyloid aggregation, protein τ hyperphosphorylation, oxidative stress and inflammation. In connection with the foregoing hypothesis of AD, there are several drug targets and strategies of treating the disease. The most popular and already registered group of drugs is AChEI inhibiting the activity of enzyme cholinesterase and increasing the level of acetylcholine in brain what have positive effects on the symptoms of AD. There are also some approaches in AD to inhibit or modulate secretases activity and reduce the generation of β-amyloid by human cells or produce less of the β-amyloid$_{42}$ and more of the shorter nontoxic peptides. A very promising line for treating AD is immunotherapy including vaccination

and immunization based on the usage of β-amyloid, β-amyloid fragments or prepared anti-β-amyloid antibodies. In connection with the inflammatory hypothesis of AD new drugs reducing inflammation are searched for. It has been observed that a long-lasting therapy with NSAIDs noticeably reduces the risk of AD. It is possible that except inhibiting COX activity there is another mechanism explaining their effectiveness in AD. Adjunctive drugs in AD treatment are natural antioxidants protecting neurons, preventing and eliminating oxidative stress such as flavonoids, polyphenols, vitamin C and E or caffeine and substances such as nicotine (agonist of nicotinic cholinergic receptors). Some positive effects in AD have also been noticed after trials of using such as statins, agonists of M1 muscarinic receptors and antagonists of NMDA receptors in AD drugs. These and other trials and efforts may cause a successful disease modifying or progression-inhibiting AD treatment in the future.

*Keywords:* Alzheimer's disease, acetylcholine, cholinesterase inhibitors, memory and learning

## STRESZCZENIE

Choroba Alzheimer'a (AD) uważana jest za najczęściej występującą chorobę otępienną. Spośród hipotez tłumaczących zanik neuronów w AD często wymienia się agregację β-amyloidu, hiperfosforylację białka τ, stres oksydacyjny oraz procesy zapalne. W oparciu o domniemane podłoże choroby opracowano szereg strategii leczenia AD, których podstawę stanowią obecnie leki z grupy inhibitorów acetylocholinoesterazy (I-AChE), które dzięki hamowaniu aktywności enzymu AChE pozwalają na zwiększenie poziomu acetylocholiny (ACh) w mózgu, co wywiera pozytywny efekt na poprawę funkcji kognitywnych. Istnieją również próby zastosowania w AD leków hamujących lub modyfikujących działanie sekretaz w celu zmniejszenia produkcji β-amyloidu, a szczególnie toksycznej formy β-amyloidu$_{42}$. Obiecującą formę leczenia AD stanowi także immunoterapia, zakładająca wprowadzenie do organizmu chorego β-amyloidu lub jego fragmentów w celu wytworzenia przeciwciał przeciwamyloidowych (immunizacja aktywna) lub też podanie gotowych przeciwciał (immunizacja pasywna). W nawiązaniu do procesów zapalnych zaobserwowanych u osób cierpiących na AD poszukuje się również nowych leków przeciwzapalnych. Stwierdzono, że długotrwałe stosowanie leków z grupy niesteroidowych leków przeciwzapalnych (NLPZ) zmniejsza ryzyko wystąpienia AD. Prawdopodobnie NLPZ poza hamowaniem aktywności cyklooksygenazy (COX) wykazują również dodatkowe działanie, tłumaczące ich efektywność w zapobieganiu AD. Wciąż badanymi lekami wspomagającymi terapię chorych na AD, z którymi wiąże się duże nadzieje, są naturalne antyoksydanty (flawonoidy, polifenole, witamina C oraz E, a także kofeina) chroniące neurony przed stresem oksydacyjnym oraz nikotyna, w stosunku do której wykazano wyraźne działanie prokognitywne. Pozytywne efekty zaobserwowano również po próbach zastosowania w AD statyn, agonistów receptora muskarynowego M1 oraz antagonistów receptora NMDA. Te oraz inne próby mogą przyczynić się do opracowania skutecznego leczenia modyfikujące przebieg AD i pozwalającego na zatrzymanie postępu choroby.

*Słowa kluczowe:* choroba Alzheimer'a, acetylocholina, inhibitory cholinesterazy, pamięć i uczenie się